# Regioselective Construction and Screening of 1,3-Disubstituted Tetrahydroindazolones in Enantiomerically Pure Pairs

Heebum Song,[†] Hanjae Lee,[†] Jonghoon Kim,[†] and Seung Bum Park*,[†,‡]

[†]Department of Chemistry and [‡]Department of Biophysics and Chemical Biology, Seoul National University, Seoul 151-747, Korea

Ⓢ *Supporting Information*

ABSTRACT: In this paper, we describe a regioselective synthetic pathway for enantiopure 1,3-disubstituted tetrahydroindazolone derivatives via the condensation of 2-acylcyclohexane-1,3-dione with various alkyl- and arylhydrazines using the steric effects of a Boc-protected pyrrolidine ring. This synthetic method has a broad scope for substrate generality for various hydrazines with excellent regioselectivity. To maximize the molecular diversity, further diversifications of 1,3-disubstituted tetrahydroindazolones were pursued by systematic *N*-modification of the secondary amine of the pyrrolidine ring using solution-phase parallel synthesis with polymer-supported reagents. A library containing a total of 272 drug-like tetrahydroindazolones, including 85 enantiomeric pairs, was constructed; the average purity, without further purification, was 95%.

A Series of Enantiomeric Pairs

KEYWORDS: *tetrahydroindazolone, regioselective synthesis, solution-phase parallel synthesis, diversity-oriented synthesis, stereochemical diversity*
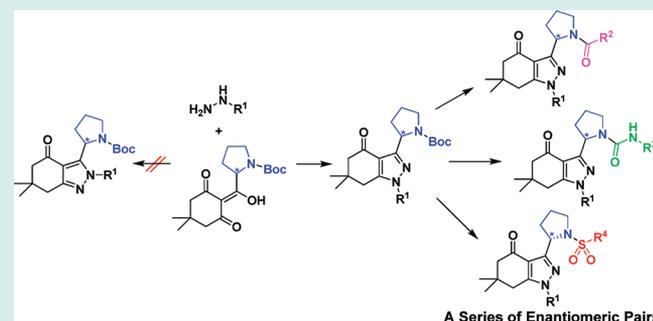
INTRODUCTION

One of the great challenges in the field of chemical biology is the identification of novel small-molecule modulators that can specifically control the functions of gene products and elucidate the associated signaling pathways.[1] In addition, small-molecule modulators can serve as perturbing agents in biomedical research to pinpoint the control of complex functions of biopolymers; this can help in the development of potential therapeutic agents.[2] For the efficient and systematic identification of novel small-molecule modulators, there is a great demand for novel and structurally diverse chemical entities.[3] The construction of drug-like small molecules with wide ranges of biological activities has therefore become an essential element of chemical biology and drug discovery.[4] To address this issue, we have been focusing on the development of divergent and robust synthetic pathways, using diversity-oriented synthesis (DOS) strategies, for the systematic construction of a library of drug-like small molecules by creative reconstruction of polyheterocycles embedded with privileged substructures including benzopyrans, pyrroles, carbohybrids, and acetal-fused pyranopyrones; we named this approach a privileged-substructure-based diversity-oriented synthesis (pDOS).[5] In addition, we have constructed various drug-like polyheterocycles, such as diazabicycles,[6a] tetrahydro-*β*-carbolines,[6b] oxopiperazines,[6c] and benzodiazepines,[6d] by developing simple and robust synthetic transformations from common cyclic iminium intermediates using a divergent approach; this provides the maximum molecular diversity with the minimum number of linear synthetic steps. Our

collection of drug-like polyheterocycles has been subjected to various biological evaluations and yielded novel small-molecule modulators such as agonists of the estrogen-related receptor *γ*,[7a] an osteogenic agent,[7b] inhibitors of RANKL-induced osteoclastogenesis,[7c] an activator of AMP-activated protein kinase,[7d] and antagonist of androgen receptors.[7e]

As a continuation of our efforts to construct novel molecular frameworks, we aimed to develop a practical and regioselective synthetic route to tetrahydroindazolone, a privileged structural motif that is frequently found in bioactive natural products and therapeutic agents.[8] As shown in Figure 1, indazole and indazolone derivatives are attractive structural motifs because of their various biological activities such as anti-inflammatory, antibacterial, and anticancer activities.[9] In particular, tetrahydroindazolone was recently recognized as an important pharmacophore because of its excellent biological activity as a potent inhibitor of heat-shock protein 90 (HSP90) and is currently in phase III clinical trials.[8,10] Despite the proven importance of tetrahydroindazolone derivatives in biomedical research, a robust and efficient synthesis of such compounds remains a challenge because of the difficulties of regiochemical control. The general procedure for regioselective synthesis of tetrahydroindazolones is a simple condensation of 2-acylcyclohexane-1,3-diones with substituted arylhydrazines. This is because of the inherent differences between the nucleophilic-
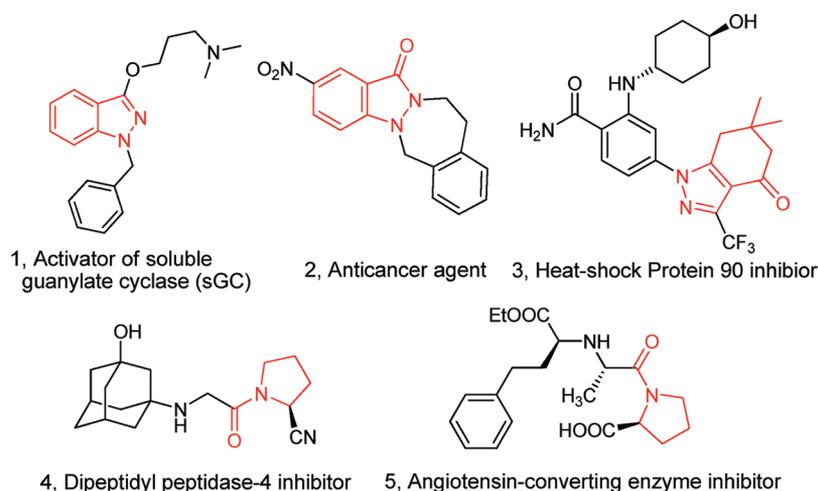
Figure 1. Biologically active compounds containing indazole, indazolone, tetrahydroindazolone, or *N*-acyl pyrrolidine moieties.

ities of the two nitrogens in arylhydrazines,[8,11] but this is not the case with alkylhydrazines.[12] To address this problem, we recently reported a regioselective pathway for the synthesis of complementary regioisomers of 1,2- and 1,3-disubstituted tetrahydroindazolones by the sequential protection and deprotection of substituted hydrazines.[13] We also reported a practical solid-phase synthetic pathway for 1,3-disubstituted tetrahydroindazolones.[14]

Herein, we report a practical pathway for the regioselective synthesis of 1,3-substituted tetrahydroindazolones with broad substrate scopes including alkylhydrazines as well as arylhydrazines. In this study, we achieved regioselectivity by the introduction of bulky *N*-Boc-pyrrolidine residues on 2-acylcyclohexane-1,3-done; this provides sufficient steric hindrance to give regioselectivity, irrespective of the substitutents on the hydrazines. In addition, the introduction of an *N*-Boc-pyrrolidine moiety on tetrahydroindazolone might enhance possible interactions with biopolymers because pyrrolidine is frequently observed in various bioactive small molecules such as antihyperglycemic agents[15a,b] and angiotensin-converting enzyme (ACE) inhibitors[15c] (Figure 1). Finally, we aimed to maximize the molecular diversity through the systematic *N*-modification on the pyrrolidine moiety and preparation of enantiomeric pairs (*R/S*) of tetrahydroindazolone derivatives, by using either L- or D-proline, for the study of stereochemical diversity.

RESULT AND DISCUSSION

Most regioselective syntheses of tetrahydroindazolone derivatives are achieved by simple condensation of 2-acylcyclohexane-1,3-diones with substituted hydrazines through the different nucleophilicities of the two nitrogens on the substituted hydrazines.[8,10,11] In the case of arylhydrazines, the internal nitrogen atom is much less nucleophilic than the external one as a result of conjugation of the lone-pair electrons on the internal nitrogen in the aromatic ring system; this leads to the regioselective synthesis of 1,3-disubustituted tetrahydroindazolones. In the case of alkylhydrazines, however, regiochemical control in the synthesis of 1,3-disubstituted tetrahydroindazolones is quite difficult to achieve because the nucleophilicities of the two nitrogens on alkylhydrazines are indistinguishable.

A different strategy is therefore needed for the robust synthesis of 1,3-disubstituted tetrahydroindazolones, that is,

one which does not depend on the native nucleophilicities of the substituted hydrazines and which has broad scope for substrate generality. First, we tested the regioisomeric ratios of simple condensations between substituted hydrazines **1** and various 2-acylcyclohexane-1,3-diones **2**. As shown in Figure 2,
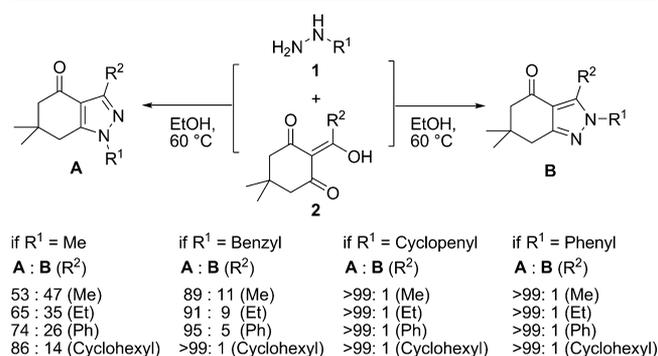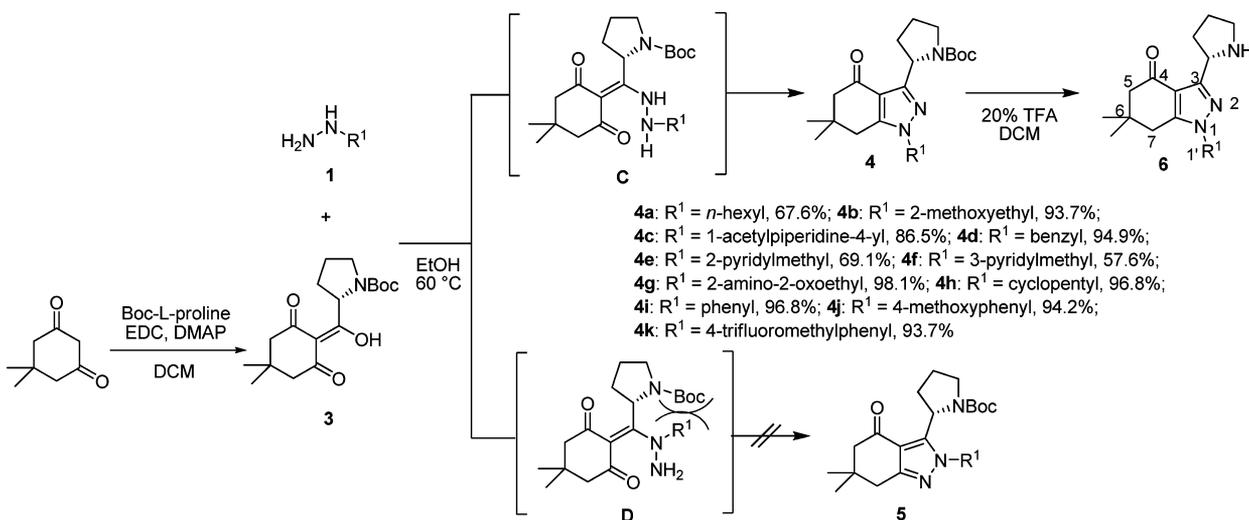

if R¹ = Me	if R¹ = Benzyl	if R¹ = Cyclopenyl	if R¹ = Phenyl
A : **B** (R²)	**A** : **B** (R²)	**A** : **B** (R²)	**A** : **B** (R²)
53 : 47 (Me)	89 : 11 (Me)	>99: 1 (Me)	>99: 1 (Me)
65 : 35 (Et)	91 : 9 (Et)	>99: 1 (Et)	>99: 1 (Et)
74 : 26 (Ph)	95 : 5 (Ph)	>99: 1 (Ph)	>99: 1 (Ph)
86 : 14 (Cyclohexyl)	>99 : 1 (Cyclohexyl)	>99: 1 (Cyclohexyl)	>99: 1 (Cyclohexyl)

Figure 2. Regioisomeric ratio [regioisomeric ratios were determined by ¹H NMR spectroscopic analysis of samples after purification via silica-gel flash column chromatography] of tetrahydroindazolones via conventional condensation of various hydrazines **1** and 2-acylcyclohexane-1,3-diones **2**.

conventional heating in protic solvents allows the formation of disubstituted tetrahydroindazolones in different regioisomeric ratios; this was monitored by ¹H and one-dimensional nuclear Overhauser effect (1D NOE) NMR. However, poor regioselectivity was observed in the case of alkylhydrazines. For example, the condensation of methylhydrazine with 2-acetylcyclohexane-1,3-dione provides an almost 1:1 mixture (53:47) of 1,3- and 1,2-disubstituted regioisomers. In this experiment, we paid particular attention to the linear correlation of the steric hindrance at the R² position on the 2-acylcyclohexane-1,3-diones **2** with regioselectivity for 1,3-disubstituted tetrahydroindazolones.[13] For a cyclohexyl moiety at the R² position on **2**, we only observed single regioisomers, except with the smallest possible substituent at the R¹ position on the substituted hydrazine, that is, methylhydrazine, in which the 1,3-isomer was preferred over the 1,2-isomer (86:14). We therefore believed that we could control the regioselectivity for 1,3-disubstituted tetrahydroindazolones, irrespective of the substituents at the R¹ position on the alkyl- or arylhydrazines,

Scheme 1. Regioselective Synthetic Pathway for 1,3-Disubstituted Tetrahydroindazolones Using Hydrazines 1 and 2-(Hydroxy(*N*-Boc-pyrrolidin-2-yl)methylene)-5,5-dimethylcyclohexane-1,3-dione 3

4a: R[1] = *n*-hexyl, 67.6%; **4b**: R[1] = 2-methoxyethyl, 93.7%;
4c: R[1] = 1-acetylpiperidine-4-yl, 86.5%; **4d**: R[1] = benzyl, 94.9%;
4e: R[1] = 2-pyridylmethyl, 69.1%; **4f**: R[1] = 3-pyridylmethyl, 57.6%;
4g: R[1] = 2-amino-2-oxoethyl, 98.1%; **4h**: R[1] = cyclopentyl, 96.8%;
4i: R[1] = phenyl, 96.8%; **4j**: R[1] = 4-methoxyphenyl, 94.2%;
4k: R[1] = 4-trifluoromethylphenyl, 93.7%

using cyclohexyl or equivalent substituents at the R[2] position on the 2-acylcyclohexane-1,3-diones. Bulkier substituents at the R[1] position on alkylhydrazines also allow some regioselectivity for the synthesis of 1,3-disubstituted tetrahydroindazolones. However, this regioselectivity is not high enough except for the limited cases such as cyclopentylhydrazines or alkylhydrazines with equivalent substituents, which makes this approach impractical for high throughput synthesis. In the case of arylhydrazines, excellent regioselectivity was obtained, irrespective of the R[2] substituents on the 2-acylcyclohexanes-1,3-diones, as expected (see full data set in the Supporting Information).

These experiments clearly showed that the condensation of substituted hydrazines **1** with 2-acylcyclohexane-1,3-diones **2** is strongly influenced by steric hindrance at the R[2] position of the 2-acylcyclohexane-1,3-diones. On the basis of these observations, we designed a regioselective synthetic pathway for 1,3-disubstituted tetrahydroindazolone derivatives by introducing a bulky substituent, namely, an *N*-Boc-protected pyrrolidine ring, at the R[2] position on 2-acylcyclohexane-1,3-diones **2** (Scheme 1). As well as achieving regioselectivity, *N*-Boc-protected pyrrolidines introduced into 1,3-disubstituted tetrahydroindazolones can be used for further *N*-modification, after Boc deprotection, to maximize the molecular diversity of the resulting small-molecule library. In addition, we might expect an improvement in the possibility of interactions with biopolymers and the subsequent discovery of bioactive small molecules because *N*-acylpyrrolidine moieties are also recognized as privileged substructures and are frequently observed in many bioactive natural products and therapeutic agents.[16]

The introduction of *N*-Boc-protected pyrrolidine on 2-acylcyclohexane-1,3-diones was achieved by 4,4-dimethylaminopyridine(DMAP)-mediated coupling of 5,5-dimethylcyclohexane-1,3-dione with Boc-protected L-proline in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC). We then validated the regioselectivity of this synthetic strategy using 2-(hydroxy(*N*-Boc-pyrrolidin-2-yl)methylene)-5,5-dimethylcyclohexane-1,3-dione **3** and benzyl hydrazine **1d**. The simple condensation of **3** at 60 °C in EtOH solution allows the regioselective formation of 1,3-disubstituted tetrahydroindazolones **4**, and was consistent for various alkylhydrazines, including *n*-hexyl (**4a**), 2-methoxyethyl (**4b**),

1-acetylpiperidin-4-yl (**4c**), and 2-pyridylmethyl (**4e**), 3-pyridylmethyl (**4f**), 2-amino-2-oxoethyl (**4g**), cyclopentyl (**4h**), as well as for arylhydraiznes (**4i**–**4k** in Scheme 1). The regiochemistry was confirmed by 1D NOE experiments after Boc deprotection in 20% trifluoroacetic acid (TFA); this demonstrated the clear correlations between protons at the C7 and C1′ positions in the 1,3-disubstituted tetrahydroindazolones **6** (see Supporting Information). We concluded that this regioselective strategy can be widely applied and has a broad scope for substrate generality at the R[1] position of hydrazines for the synthesis of 1,3-disubstituted tetrahydroindazolones.

After establishing the regioselective synthetic pathway for 1,3-disubstituted tetrahydroindazolones with a broad scope for substituted hydrazines, we focused on further diversification of tetrahydroindazolones as key intermediates for maximizing molecular diversity. As shown in Scheme 2, the introduction of

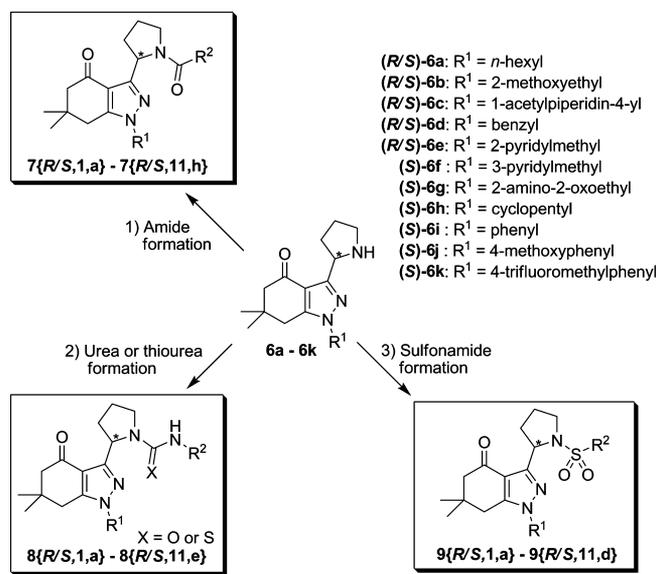## Scheme 2. Further Diversification Strategy for 1,3-Disubstituted Tetrahydroindazolones

(*R/S*)-**6a**: R[1] = *n*-hexyl
(*R/S*)-**6b**: R[1] = 2-methoxyethyl
(*R/S*)-**6c**: R[1] = 1-acetylpiperidin-4-yl
(*R/S*)-**6d**: R[1] = benzyl
(*R/S*)-**6e**: R[1] = 2-pyridylmethyl
(*S*)-**6f**: R[1] = 3-pyridylmethyl
(*S*)-**6g**: R[1] = 2-amino-2-oxoethyl
(*S*)-**6h**: R[1] = cyclopentyl
(*S*)-**6i**: R[1] = phenyl
(*S*)-**6j**: R[1] = 4-methoxyphenyl
(*S*)-**6k**: R[1] = 4-trifluoromethylphenyl

Table 1. Building Blocks and Purities[a] of 7{R/S,R^1,R^2} Produced via N-modification (I): Amide Formation with Activated Esters on HOBt Resin Using Solution-Phase Parallel Synthesis

		7{R/S,R^1,R^2}	R^2	a	b	c	d	e	f	g	h
7{S,R^1,R^2}	R^1	1	n-Hexyl		99.5	86.8	85.3	97.6	93.8	93.8	93.2	88.8
		2	2-Methoxyethyl		99.6	96.5	97.9	99.6	99.5	99.7	98.6	98.8
		3	1-Acetylpiperidin-4-yl		93.1	84.7	84.0	95.5	95.5	96.5	97.4	97.4
		4	Benzyl		95.3	93.0	96.9	98.5	90.3	98.8	99.7	90.8
		5	2-Pyridylmethyl		99.4	98.7	99.18	98.5	96.8	99.2	96.4	94.9
7{R,R^1,R^2}	R^1	1	n-Hexyl		97.1	92.2	98.2	99.8	98.9	98.2	99.0	99.0
		2	2-Methoxyethyl		99.5	96.6	96.0	97.7	98.3	97.6	96.7	99.2
		3	1-Acetylpiperidin-4-yl		95.6	87.6	85.6	97.5	98.6	97.0	94.1	94.2
		4	Benzyl		92.5	92.1	94.8	98.1	92.9	93.6	96.5	97.4
		5	2-Pyridylmethyl		98.3	99.2	92.4	98.7	96.9	97.8	97.6	98.3
7{S,R^1,R^2}	R^1	6	3-Pyridylmethyl		98.3	98.2	96.0	98.0	98.0	95.2	99.4	95.7
		7	2-Amino-2-oxoethyl		90.1	95.9	97.6	98.3	97.4	95.9	91.7	94.3
		8	Cyclopentyl		97.8	89.9	82.5	98.0	92.9	98.1	96.7	97.2
		9	Phenyl		99.6	94.7	81.8	92.1	95.7	94.4	95.6	93.7
		10	4-Methoxyphenyl		98.7	98.2	89.8	99.0	98.2	98.4	94.9	93.5
		11	4-Trifluoromethylphenyl		92.7	93.6	93.8	95.4	93.9	90.2	96.0	93.0

[a]Purities were obtained by PDA-based LC/MS analysis of final products without further purification.

various substituents at the R^1 position can be achieved using various hydrazines, including alkyl- and arylhydrazines. The resulting tetrahydroindazolone derivatives were further diversified through N-modification at the pyrrolidine secondary amine as follows: (i) amide formation with various carboxylic acids, (ii) urea or thiourea formation with isocyanates or isothiocyanates, and (iii) sulfonamide formation with sulfonyl chlorides (Scheme 2). For convenient and efficient N-modification, we adopted solution-phase parallel synthesis using polymer-supported reagents.

First, amide formation was achieved by direct incubation of the free secondary amine on the pyrrolidine moiety of tetrahydroindazolones 6 in dicholoroethane (DCE) for 24 h at room temperature with activated esters on an HOBt-6-carboxaminomethyl polystyrene support generated by treatment of carboxylic acid in the presence of N,N'-diisopropyl-carbodiimide (DIC), DMAP, and diisopropylethylamine (DIPEA) in dimethylformamide (DMF) for 4 h at room temperature. As shown in Table 1, we successfully generated 128 tetrahydroindazolone derivatives 7 via pyrrolidine modification of 16 different tetrahydroindazolones 6, with amide formation using eight different carboxylic acids that included alkyl, aryl, acrylic, and heterocyclic substitutents. The resulting compounds 7 had excellent purities without further purification; the overall purity was 95.6% (Table 1).

Urea/thiourea formation and sulfonamide formation were also pursued by solution-phase parallel synthesis using polymer-

supported scavengers to remove excess N-modification reagents. The 1,3-disubstituted tetrahydroindazolone intermediates 6 were treated with an excess of isocyanates or isothiocyanates and sulfonyl chlorides in DCE containing triethylamine (TEA) at room temperature; this provided the desired ureas or thioureas and sulfonamides, respectively. Excess N-modification reagents were then removed by *tris*-(2-aminoethyl)amine (TAEA) polystyrene scavenger resin in DCE for 24 h at room temperature. Initially, we pursued the N-modification of pyrrolidines with isocyanates or isothiocyanates in the absence of an organic base; this required a higher temperature (80 °C) and a longer time (12−24 h) to achieve full conversion of the starting materials. These initial conditions with an elevated temperature gave moderate yields, and an undesired aza-Cope rearrangement was observed in the case of allyl isothiocyanate (data not shown); this led us to N-modification in the presence of an organic base under much milder conditions (2.5 h at room temperature). As shown in Table 2, we successfully generated 80 tetrahydroindazolone derivatives 8 with urea or thiourea moieties by modification with three different isocyates and two different isothiocyanates, respectively, with an overall purity of 95.5%.

In the case of sulfonamide formation, we successfully generated 64 1,3-disubstituted tetrahydroindazolones 9 with sulfonamide moieties, with an overall purity of 93.7%, without further purification, by treatment of four different sulfonyl chlorides in the presence of TEA at room temperature,

Table 2. Building Blocks and Purities[a] of 8{R/S,R[1],R[2]} Produced via N-modification (II): Urea/Thiourea Formation with Isocyanates/Isothiocyanates and Subsequent Scavenging with Trisamine Resin

		8{R/S,R[1],R[2]}	R[2]	a	b	c	d	e
8{S,R[1],R[2]}	1	n-Hexyl	R[1]	92.3	95.6	92.2	92.2	98.6
	2	2-Methoxyethyl		94.4	97.6	98.0	97.8	97.8
	3	1-Acetylpiperidin-4-yl		99.4	96.9	93.0	97.0	96.1
	4	Benzyl		91.3	97.0	94.7	98.2	96.8
	5	2-Pyridylmethyl		92.2	93.9	89.6	99.5	99.4
8{R,R[1],R[2]}	1	n-Hexyl	R[1]	98.4	94.7	93.0	95.4	96.1
	2	2-Methoxyethyl		95.8	98.7	98.7	95.7	94.3
	3	1-Acetylpiperidin-4-yl		99.2	95.5	98.6	95.3	93.9
	4	Benzyl		96.3	96.0	96.5	92.6	97.0
	5	2-Pyridylmethyl		98.3	99.5	99.4	99.9	99.4
8{S,R[1],R[2]}	6	3-Pyridylmethyl	R[1]	94.6	92.1	93.9	98.6	97.1
	7	2-Amino-2-oxoethyl		91.7	95.8	90.0	96.6	97.0
	8	Cyclopentyl		91.2	95.2	94.3	97.4	97.9
	9	Phenyl		94.9	97.9	96.9	90.7	97.9
	10	4-Methoxyphenyl		95.9	95.3	97.2	95.8	98.4
	11	4-Trifluoromethylphenyl		91.4	90.0	84.6	95.8	87.0

[a]Purities were obtained by PDA-based LC/MS analysis of final products without further purification.

followed by removal of excess reagents via incubation with TAEA polystyrene resins for 24 h at room temperature (Table 3).

It is worth mentioning that we constructed a 1,3-disubstituted tetrahydroindazolone library containing a series of enantiomeric pairs, synthesized as a single enantiomer, because we believe that stereochemical diversity is relatively unexplored among the diversity elements in the DOS approach, and that the systematic construction of enantiomeric pairs might provide information regarding the importance of stereochemical outcomes in molecular interactions with various biopolymers. In fact, most biopolymers, including proteins, DNA, RNA, and polysaccharides, are in chiral environments and they should have clear preferences for a particular stereochemistry of their small-molecule partners. Once we can access a systematic collection of enantiomeric pairs having identical physical and chemical properties, we can explore the importance of a single stereogenic center in biological environments through various bioevaluations. There are sporadic reports in the literature,[17] but systematic studies are still rare. To address this unmet need, we constructed a library of enantiopure 1,3-disubstituted tetrahydroindazolones; the library contained 40 enantiomeric pairs from 7{S,1,a} to 7{R,5,h}, 25 pairs from 8{S,1,a} to 8{R,5,e}, and 20 pairs from 9{S,1,a} to 9{R,5,d}. The (S) enantiomer sets were derived from L-proline for the synthesis of 2-(hydroxy(N-Boc-pyrrolidin-2-yl)methylene)-5,5-dimethylcyclohexane-1,3-dione 3, and the (R) enantiomer sets were derived from D-proline for the synthesis of the key intermediates 3.

The resulting 272-membered tetrahydroinazolone library with 85 enantiomeric pairs was subjected to biological evaluation to seek potential activities based on their structures and stereochemistries. In this context, our tetrahydroindazolone derivatives were treated against HeLa human cervical cancer cells. The compound-induced inhibition of cell proliferation was measured by monitoring mitochondrial activity with 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate (WST-1, cell proliferation assay reagent) upon treatment with the compounds in the concentration range 100 nM to 20 μM for 72 h. As show in Figure 3, we observed interesting patterns of antiproliferative activity toward HeLa cells among the enantiomer sets. For example, the enantiomeric pair, 8{R,1,e} and 8{S,1,e}, show similar antiproliferative activities (IC$_{50}$ = 6.64 μM and 6.71 μM, respectively). On the other hand, a different enantiomeric pair, 8{R,1,d} and 8{S,1,d}, show totally different activities: compound 8{R,1,d} shows some cytotoxicity toward HeLa cells (IC$_{50}$ = 6.51 μM), but the enantiomer 8{S,1,d} has no cytotoxic activity, even at the highest treatment concentration (20 μM). This observation indicates the importance of stereochemical diversity, and that we might be missing much information regarding dependence on compound chirality in various biological evaluations (see Supporting Information for viability data of whole library compounds).

■ CONCLUSION

In this study, we developed a practical and robust strategy for regioselective synthesis of 1,3-disubstituted tetrahydroindazo-lone derivatives by introduction of steric hindrance using a Boc-

Table 3. Building Blocks and Purities[a] of 9{R/S,R¹,R²} Produced via N-modification (III): Sulfonamide Formation Using Sulfonyl Chlorides and Subsequent Scavenging with Trisamine Resin

	9{R/S,R¹,R²}			R²	a	b	c	d
9{S,R¹,R²}	R¹	1	n-Hexyl		90.5	90.5	92.1	92.2
		2	2-Methoxyethyl		93.1	92.7	94.3	83.6
		3	1-Acetylpiperidin-4-yl		95.1	99.0	95.4	86.6
		4	Benzyl		94.9	96.9	93.0	92.1
		5	2-Pyridylmethyl		91.6	93.2	98.2	97.6
9{R,R¹,R²}	R¹	1	n-Hexyl		90.8	90.1	90.1	87.1
		2	2-Methoxyethyl		94.5	94.6	97.8	92.8
		3	1-Acetylpiperidin-4-yl		92.2	96.1	88.2	94.8
		4	Benzyl		96.4	98.8	97.7	92.5
		5	2-Pyridylmethyl		98.2	96.8	98.8	97.3
9{S,R¹,R²}	R¹	6	3-Pyridylmethyl		88.2	92.5	98.9	89.1
		7	2-Amino-2-oxoethyl		88.2	93.9	95.5	89.4
		8	Cyclopentyl		93.4	91.7	90.9	92.3
		9	Phenyl		94.1	94.0	91.1	93.5
		10	4-Methoxyphenyl		97.1	96.7	96.2	95.6
		11	4-Trifluoromethylphenyl		97.4	98.0	96.3	92.4

[a]Purities were obtained by PDA-based LC/MS analysis of final products without further purification.
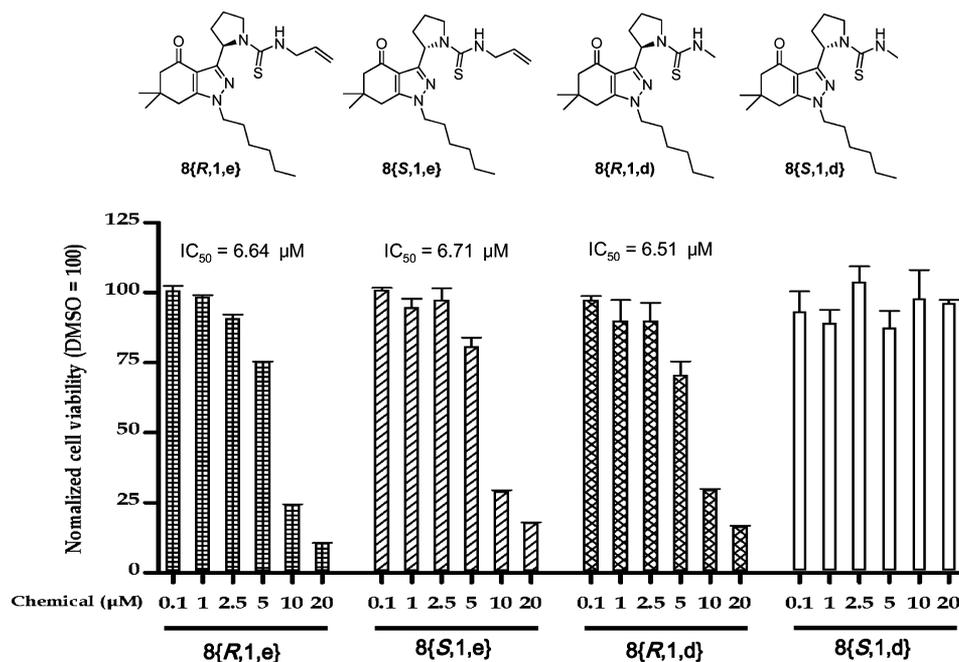

Figure 3. Representative enantiomeric compound sets and their antiproliferative activities [cell proliferative data was measured by WST-1 assay upon treatment with representative compounds against HeLa cell in does-dependent pattern for 72 h. All experiments were duplicated and mean value was used] against HeLa human cervical cancer cells.

protected pyrrolidine ring on 2-acylcyclohexane-1,3-diones **2**. As a result of this steric hindrance, we successfully controlled the regioselectivity of 1,3-disubstituted tetrahydroindazolones

with a broad scope for substrate generality for substituted hydrazines, including alkylhydrazines. The pyrrolidine moiety can also serve as a privileged substructure in the resulting

tetrahydroindazolone derivatives; this might increase the probability of potential interactions with various biopolymers for the discovery of novel bioactive small molecules. We particularly focused on constructing a library of enantiopure tetrahydroindazolone derivatives and the preparation of 85 enantiomer pairs, simply by the introduction of chiral pyrrolidine from L-/D-proline. To maximize the molecular diversity and process efficiency, we pursued the systematic N-modification of pyrrolidine secondary amine using solution-phase parallel synthesis in conjunction with polymer-supported reagents. Finally, we constructed a 272-membered 1,3-disubstituted tetrahydroindazolone library containing 85 enantiomeric pairs, without further purification, with an average purity of 95.1%. In our preliminary biological evaluations, a series of enantiopure tetrahydroindazolone derivatives showed dramatic differences in their antiproliferative activity toward HeLa cells, depending on the differences in their stereo-chemistries; this emphasizes the importance of stereochemical diversity in library construction using combinatorial chemistry or diversity-oriented synthesis. Further biological evaluations of the resulting tetrahydroindazolones will be reported in due course.

■ EXPERIMENTAL PROCEDURES

General Information. All commercially available reagents and solvents were used without further purification unless noted otherwise. All solvents were purchased from commercial suppliers. HOBt-6-carboxamiomethyl polystyrene (PS-HOBt) and tris-(2-aminoethyl)-amine polystyrene (PS-trisamine) were purchased from Novabiochem. Analytical thin-layer chromatography (TLC) was performed using 0.25-mm silica-gel-coated Kiselgel 60 F_{254} plates, and the components were visualized by observation under UV light (254 and 365 nm) or by treating the plates with ninhydrin followed by thermal visualization. ^{1}H, ^{13}C, and 1D NOE NMR spectra were obtained using a Varian Inova-500 (Varian, Palo Alto, CA, U.S.A.). Chemical shifts were reported in parts per million from tetramethylsilane (TMS) as internal standard or the residual solvent peak (CDCl$_3$, ^{1}H: 7.26, ^{13}C: 77.23; (CD$_3$)$_2$SO, ^{1}H: 2.50, ^{13}C: 39.52). Multiplicities were indicated as follows: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublets); dt (doublet of triplets); td (triplet of doublets); bs (broad singlet). Coupling constants were reported in hertz. The purities of all the library members were determined using an LC/MS system equipped with a reverse-phase column (C-18, 50 × 2.1 mm, 5 μm) and a photodiode array (PDA) detector using electron spray ionization (ESI).

tert-Butyl 2-((4,4-dimethyl-2,6-dioxocyclohexylidene)-(hydroxy)methyl)pyrrolidine-1-carboxylate (3). To a solution of (2S)-1-(tert-butoxycarbonyl)-2-pyrrolidine carboxylic acid (N-Boc-L-proline) or (2R)-1-(tert-butoxycarbonyl)-2-pyr-rolidinecarboxylic acid (N-Boc-D-proline) (1.2 equiv.), 4-dimethylaminopyridine (DMAP) (1.0 equiv.), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (1.3 equiv.) in anhydrous dichloromethane (DCM) (0.1 M), dimedone (1.0 equiv.) was added, and the reaction mixture was stirred for several hours under an argon atmosphere. After complete consumption of the starting materials, monitored by TLC, the reaction mixture was diluted with ethyl acetate (EA) and washed twice with saturated NH$_4$Cl (aq). The combined organic layer was dried over anhydrous Na$_2$SO$_4$, filtered, and condensed under reduced pressure. Silica-gel flash column chromatography provided the desired product **3** (68.4%) as a mixture of rotamers (43:57).

General Synthetic Procedure for (S)-6,6-dimethyl-3-(N-Boc-pyrrolidin-2-yl)-1,3-disubstituted Tetrahydoin-dazolones (4). To a 0.1 M solution of (S)-tert-butyl-2-((4,4-dimethyl-2,6-dioxocyclohexylidene)(hydroxy)methyl)-pyrrolidine-1-carboxylate **3** (1.0 equiv.) in ethanol, hydrazine **1** (1.2 equiv.) was added, and the resulting mixture was stirred at 60 °C. After complete consumption of the starting materials, the reaction mixture was concentrated under reduced pressure and purified by silica-gel flash column chromatography to give the desired products **4**.

General Synthetic Procedure for (S)-6,6-dimethyl-3-(pyrrolidin-2-yl)-1,3-disubstitued Tetrahydroindazolone (6). 0.1 M solution of (S)-6,6-dimethyl-3-(N-Boc-pyrrolidin-2-yl)-1,3-disubstituted tetrahydroindazolone **4** in 20% trifluoro-acetic acid in DCM was stirred at room temperature. After complete consumption of the starting materials, monitored by TLC, the reaction was quenched by the addition of saturated NaHCO$_3$ solution and extracted three times with DCM. The combined organic layer was dried over Na$_2$SO$_4$(s). The filtrate was condensed under reduced pressure and purified by silica-gel flash column chromatography to provide **6**.

General Procedure for the Solution-Phase Parallel Synthesis of Compounds 7, 8, and 9. *A. Amide Formation for 7{S,1,a}–7{S,11,h} (128 Compounds).* PS-HOBt resin (90 mg, loading level 1.1 g/mol) was loaded in individual wells of a 96-deep-well filtration block. Eight different carboxylic acids (3.5 equiv.) in 0.6 mL of a DMF solution of N,N′-diisopropylcarbodiimide (7.0 equiv.), diiso-propylethylamine (7.0 equiv.), and 4-dimethylaminopyridine (0.2 equiv.) were added to the designated wells of the reaction block. The reaction block was sealed and incubated for 4 h at room temperature in a rotating oven (Robbins Scientific). The resulting resin was thoroughly washed four times with dichloromethane. Then, 16 Boc-deprotected tetrahydroindazo-lone intermediates **6** (11 mg) in 0.6 mL of DMF were added to the designated wells charged with individual activated esters on PS-HOBt resin. The reaction block was sealed and incubated for 24 h at room temperature in a rotating oven. The final products were collected by removal of excess activated-ester-bound PS-HOBt resins via filtration and condensing the filtrate *in vacuo* using a GeneVac (Thermo Savant). The crude products (7{S,1,a}–7{S,11,h}) were directly analyzed by LC/MS without further purification.

B. Urea and Thiourea Formation for 8{S,1,a}–8{S,11,e} (80 Compounds). Sixteen different Boc-deprotected tetrahy-droindazolone intermediates **6** (11 mg) and triethylamine (2 equiv.) were dissolved in 1,2-dichloroethane (0.3 mL) and added to a 96-deep-well filtration block. Three isocyanates and two isothiocyanates were added to the designated wells of the reaction block. The reaction block was sealed and incubated for 2.5 h at room temperature in a rotating oven. After completion of the reaction, *tris*(2-aminoethyl)amine polystyrene scavenger resin (6.0 equiv., loading level ≥2.2 mmol/g) was added to each well and incubated for an additional 24 h at room temperature. After removal by filtration of the tris(2-aminoethyl)amine polystyrene resin, the filtrate was condensed in vacuo using a GeneVac. The crude products (8{S,1,a}–8{S,11,e}) were directly analyzed by LC/MS without further purification.

C. Sulfonamide Formation for 9{S,1,a}–9{S,11,d} (64 Compounds). Sixteen different Boc-deprotected tetrahydroin-

dazolone intermediates **6** (11 mg) in a solution of 1,2-dichloroethane (0.4 mL) containing triethylamine (1.5 equiv.) were dispensed into a 96-deep-well filtration block. Four different sulfonyl chlorides were added to the designated wells, and the reaction block was sealed and incubated for 2 h at room temperature in a rotating oven. After completion of the reaction, tris(2-aminoethyl)amine polystyrene scavenger resin (10.0 equiv., loading level ≥2.2 mmol/g) was added to each well and incubated for an additional 24 h at room temperature. After removal by filtration of the tris(2-aminoethyl)amine polystyrene resin, the filtrate was condensed in vacuo using a GeneVac. The crude products (**9**{*S*,**1,a**}−**9**{*S*,**11,d**}) were directly analyzed by LC/MS without further purification.

Cell Proliferation Assay. HeLa human cervical cancer cells were cultured in RPMI 1640 media containing 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution. HeLa cells (2000 cells per well) in a 96-well plate were incubated at 37 °C in an atmosphere of 5% CO_2 and 95% air for 72 h. The cells were treated with various concentrations of individual compounds from 1,3-disubstituted tetrahydroindazolone library using pin tool. After 72 h incubation, 10 μL of EZ-Cytox cell viability assay kit solution (premixed WST-1 cell proliferation assay reagent, Daeil lab service, Seoul, Korea) was added to each well and incubated for additional 1 h. The reduction of tetrazolium salts to formazan by the inhibition of metabolic activity in mitochondria was quantified by measuring the absorbance at 455 nm using Synergy HT multimode microplate reader.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H/^{13}C NMR and 1D NOE spectra to confirm the regioselectivity of compounds in Figure 2 and compounds **6a**−**6k**, full characterization of representative compounds, and LC/MS analysis and cell proliferative data of all library members. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*E-mail: sbpark@snu.ac.kr.

■ REFERENCES

(1) (a) Schreiber, S. L. Small molecules: the missing link in the central dogma. *Nat. Chem. Biol.* **2005**, *1*, 64−66. (b) Hopkins, A. L.; Groom, C. R. The druggable genome. *Nat. Rev. Drug Discovery* **2002**, *1*, 727−730. (c) Strausberg, R. L.; Schreiber, S. L. From knowing to controlling: a path from genomics to drugs using small molecule probes. *Science* **2003**, *300*, 294−295.

(2) (a) Schreiber, S. L. Chemical genetics resulting from a passion for synthetic organic chemistry. *Bioorg. Med. Chem.* **1998**, *6*, 1127−1152. (b) Spring, D. R. Diversity-oriented synthesis; a challenge for synthetic chemists. *Org. Biomol. Chem.* **2003**, *1*, 3867−3870. (c) Tan., D. S. Diversity-oriented synthesis: exploring the intersections between chemistry and biology. *Nat. Chem. Biol.* **2005**, *1*, 74−84. (d) Spandl,

R. J.; Bender, A.; Spring, D. R. Diversity-oriented synthesis; a spectrum of approaches and results. *Org. Biomol. Chem.* **2008**, *6*, 1149−1158. (e) Peuchmaur, M.; Y.-S. Wong., Y.-S. Expanding the chemical space in practice: diversity-oriented synthesis. *Comb. Chem. High Throughput Screening* **2008**, *11*, 587−60.

(3) Lombardino, J. G.; Lowe, J. A. III. The role of the medicinal chemist in drug discovery-Then and now. *Nat. Rev. Drug Discovery* **2004**, *3*, 853−862.

(4) Burke, M. D.; Schreiber, S. L. A planning strategy for diversity-oriented synthesis. *Angew. Chem., Int. Ed.* **2004**, *43*, 46−58.

(5) Oh, S.; Park, S. B. A design strategy for drug-like polyheterocycles with privileged substructures for discovery of specific small-molecule modulators. *Chem. Commun.*, published online Aug 25, **2011**; DOI: 10.1039/C1CC14042F.

(6) (a) Lee, S.-C.; Park, S. B. Efficient solid-phase parallel synthesis of natural product-lkie diaza-bridged heterocycles through pictet-spengler intramolecular cyclization. *J. Comb. Chem.* **2006**, *8*, 50−57. (b) Lee, S.-C.; Choi, S. Y.; Chung, Y. K.; Park, S. B. Preparation of pilot library with tetrahydro-*β*-carboline alkaloid core skeleton using tandem intramolecular pictet-spengler cyclization. *Tetrahedron Lett.* **2006**, *47*, 6843−6847. (c) Lee, S.-C.; Park, S. B. Practical solid-phase parallel synthesis of 5−2-oxopiperazines via N-acyliminium ion cyclization. *J. Comb. Chem.* **2007**, *9*, 828−835. (d) Lee, S.-C.; Park, S. B. Novel application of leuckart-wallach reaction for synthesis of tetrahydro-1,4-benzodiazepin-5-ones library. *Chem. Commun.* **2007**, 3714−3716.

(7) (a) Koh, M.; Park, S. B. Computer-aided design and synthesis of tetra-aryl-substituted alkenes and their bioevaluation as a selective modulator of estrogen-related receptor g. *Mol. Diversity* **2011**, *15*, 69−81. (b) Oh, S.; Cho, S. W.; Yang, J. Y.; Snu, H. J.; Chung, Y. S.; Shin, C. S.; Park, S. B. Discovery of a novel benzopyranyl compound as a potent in vitro and in vivo osteogenic agent. *Med. Chem. Commun.* **2011**, *2*, 76−80. (c) Zhu, M.; Kim, M. H.; Lee, S.; Kim, S. H.; Park, S. B. Discovery of novel benzopyranyl tetracycles that act as inhibitors of RANKL-induced osteoclastogenesis. *J. Med. Chem.* **2010**, *53*, 8760−8764. (d) Oh, S.; Kim, S. J.; Hwang, J. H.; Lee., H. Y.; Ryu, M. J.; Park, J.; Kim, S. J.; Jo, Y. S.; Kim, Y. K.; Lee, C. H.; Kweon, K. R.; Shong, M.; Park, S. B. Antidiabetic and antiobesity effects of ampkinone (6f), a novel small molecule activator of AMP-activated protein kinase. *J. Med. Chem.* **2010**, *53*, 7405−7413. (e) Oh, S.; Nam, H. J.; Park, J.; Beak, S. H.; Park, S. B. Development of a novel benzopyran-containing androgen receptor antagonist to treat antiandrogen-resistant prostate cancer. *ChemMedChem* **2010**, *5*, 529−533.

(8) Barta, T. Te.; Veal, J. M.; Rice, J. W.; Partridge, J. M.; Fadden, R. P.; Ma, W.; Jenks, M.; Geng, L.; Hanson, G. J.; Huang, K. J.; Barabasz, A. F.; Foley, B. E.; Otto, J.; Hall, S. E. Discovery of benzamide tetrahydro-4*H*-carbazol-4-ones as novel small molecule inhibitors of Hsp90. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3517−3521.

(9) (a) Lien, J. C.; Lee, F. Y.; Huang, L. J.; Pan, S. L.; Guh, J. H.; Teng, C. M.; Kuo, S. C. 1-benzyl-3-(5′hydroxymethyl-2′furyl)-indazole (YC-1) derivatives as novel inhibitors against sodium nitroprusside-induced apoptosis. *J. Med. Chem.* **2002**, *45*, 4947−4949. (b) Schindler, R.; Fleischhauser, I.; Höfgen, N.; Sauer, W.; Egerland, U.; Poppe, H.; Heer, S.; Szelenyi, I.; Kutscher, B.; Engel, J. 1,5-Disubstituted indazol-3,-ols with anti-inflammatory activity. *Arch. Pharm. Pharm. Med. Chem.* **1998**, *331*, 13−21. (c) Boehringer, M.; Boehm, H. J.; Bur, D.; Gmuender, H.; Huber, W.; Klaus, W.; Kostrewa, D.; Kuehne, H.; Luebbers, T.; Meunier-Keller, N.; Mueller, F. Novel inhibitors of DNA gyrase: 3D structure based biased needle screening, hit validation by biophysical methods, and 3D guided optimization. A promising alternative to random screening. *J. Med. Chem.* **2000**, *43*, 2664−2674. (d) Cerecetto, H.; Gerpe, A.; Gonzalez, M.; Aran, V. J.; de Ocariz, C. O. Pharmacological properties of inadazole derivatives: recent developments. *Mini-Rev. Med. Chem.* **2005**, *5*, 869−878.

(10) (a) Huang, K. H.; Veal, J. M.; Fadden, R. P.; Rice, J. W.; Eaves, J.; Strachan, J.-P.; Barabasz, A. F.; Foley, B. E.; Barta, T. E.; Ma, W.; Silinski, M. A.; Hu, M.; Partridge, J. M.; Scott, A.; DuBois, L. G.; Freed, T.; Steed, P. M.; Ommen, A. J.; Smith, E. D.; Hughes, P. F.; Woodward, A. R.; Hanson, G. J.; McCall, W. S.; Markworth, C. J.; Hinkley, L.; Jenks, M.; Geng, L.; Lewis, M.; Otto, J.; Pronk, B.;

Verleysen, K.; Hall, S. E. Discovery of novel 2-aminobenzamide inhibitors of heat shock protein 90 as potent, selective and orally active antitumor agents. *J. Med. Chem.* **2009**, *52*, 4288−4305. (b) Chandarlapaty, S.; Sawai, A.; Ye, Q.; Scott, A.; Silinski, M.; Huang, K.; Fadden, P.; Partdrige, J.; Hall, S.; Steed, P. SNX2112, a synthetic heat shock protein 90 inhibitor, has potent antitumor activity against HER kinase-dependent cancers. *Clin. Cancer Res.* **2008**, *14*, 240−248. (c) Okawa, Y.; Hideshima, T.; Steed, P.; Vallet, S.; Hall, S.; Huang, K.; Rice, J.; Barabasz, A.; Foley, B.; Ikeda, H. SNX-2112, a selective Hsp90 inhibitor, potently inhibits tumor cell growth, angiogenesis, and osteoclastogenesis in multiple myeloma and other hematologic tumors by abrogating signaling via Akt and ERK. *Blood* **2009**, *113*, 846−855.

(11) (a) Rubinov, D. B.; Rubinova, I. L.; Akhrem, A. A. Chemistry of 2-acylcycloalkane-1,3-diones. *Chem. Rev* **1999**, *99*, 1047−1066. (b) Khlebnicova, T. S.; Isakova, V. G.; Lakhvich, F. A.; Kurman, P. V. Regioselective synthesis of fluorine-containing indazolones from 2-acylcyclohexane-1,3-diones. *Chem. Heterocycl. Compd.* **2008**, *44*, 301−308.

(12) (a) Schenon, P.; Mosti, L.; Menozzi, G. Reaction of 2-dimethylaminomethylene-1,3-diones with dinucleophiles. I. Synthesis of 1,5-disubstituted 4-acylpyrazoles. *J. Heterocycl. Chem.* **1982**, *19*, 1355−1361. (b) Molteni, V.; Hamilton, M. M.; Mao, L.; Crane, C. M.; Termin, A. P.; Wilson, D. M. Aqueous one-pot synthesis of pyrazoles, pyrimidines and isoxazoles promoted by microwave irradiation. *Synthesis* **2002**, 1669−1674. (c) Sucrow, W.; Mentzel, C.; Slopianka, M. Weitere enhydrazone, tetrahydroindazolone und enhydrazine aus cyclischen *β*-dicarbonylverbindungen. *Chem. Ber.* **1973**, *106*, 450−459. (d) Middleton, R. J.; Mellor, S. L.; Chhabra, S. R.; Bycroft, B. W.; Chan, W. C. Expedient synthesis of a novel class of pseudoaromatic amino acids: tetrahydroindazol-3-yl and tetrahydrobenzisozazol-3-ylalanine derivatives. *Tetrahedron Lett.* **2004**, *45*, 1237−1242.

(13) Kim, J.; Song, H.; Park, S. B. Orthogonal regioselective synthesis of *N*-alkyl-3-substituted tetrahydroindazolones. *Eur. J. Org. Chem.* **2010**, *20*, 3815−3822.

(14) Kim, J.; Song, H.; Park, S. B. Solid-phase parallel synthesis of a tetrahydroindazolone library containing tree unique core skeletons. *Chem.—Asian. J.* **2011**, *17*, 4905−4913.

(15) (a) Ahrén, B.; Landin-Olsson, M.; Jansson, P.-A.; Svensson, M.; Holmes, D.; Schwe, A. Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains lnsulin levels, and reduces glucagon level in type 2 diabetes. *J. Clin. Endocrinol. Metab.* **2004**, *89*, 2078−2084. (b) Mentlein, R.; Gallwitz, B.; Schmidt, W. E. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7−36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur. J. Biochem.* **1993**, *214*, 829−835. (c) McMurrary, J. J. V. Systolic heart failure. *N. Engl. J. Med.* **2010**, 228−238.

(16) (a) Horton, D. A.; Bourne, G. T.; Smythe, M, L. The combinatorial synthesis of bicyclic privileged structures or privileged substructures. *Chem. Rev.* **2003**, *103*, 893−930. (b) Tan, B.; Zeng, X.; Lu, Y.; Chua, P. J.; Zhong, G. Rational design of organocatalyst: highly stereoselective Michael addition of cyclic ketones to nitroolefins. *Org. Lett.* **2009**, *11*, 1927−1930.

(17) (a) Roth, F. E.; Govier, W. M. Comparative pharmacology of chlorpheniramine (chlortrimeton) and its optical isomers. *J. Pharmacol. Exp. Ther.* **1958**, *127*, 347−349. (b) Jacobson, A. E. In *Problems of drug dependence 1989*; Harris, L. S., Ed.; U.S. Government Printing Office: Washington, DC, 1989; p 556. (c) Ito, T.; Ando, H.; Suzuki, T.; Ogura, T.; Hotta, K.; Imamura, Y.; Yamaguchi, Y.; Handa, H. Identification of a primary target of thalidomide teratogenicity. *Science* **2010**, *327*, 1345−1350.