Efficient Discovery of Selective Small Molecule Agonists of Estrogen-Related Receptor γ using Combinatorial Approach

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With the goal of discovering a selective agonist of estrogen-related receptor γ (ERRγ) with enhanced potency, we designed a series of small-molecule ligands derived from a known ERRγ agonist, GSK4716, that can substantially potentiate the transcriptional activity of ERRγ. Individual compounds among a 30-member library of acyl hydrazones were pre-evaluated through in silico docking studies on the receptor cavities of ERRγ LBDs using X-ray crystal structures cocrystallized with GSK4716 and 4-OHT. This rational approach to achieve the enhanced potency in ERRγ transcriptional activity with selectivity over ERRα/β enables us to complete the construction of a focused library by carrying out microwave-assisted parallel synthesis with excellent yields and purities. Finally, we identified a more potent ERRγ agonist, **E6**, with excellent selectivity over ERRα/β.

Introduction

Nuclear receptors (NRs) play pivotal roles in activating gene transcription for the embryonic development, growth, differentiation, metabolism, reproduction, homeostasis, and morphogenesis of higher organisms and humans.[1] Most NR ligands are hydrophobic, lipid-soluble, and small in size.[2] Endogenous ligands for NRs include various cholesterol derivatives, such as steroid hormones, vitamin D, bile acids, retinoids (active forms of vitamin A), and thyroid hormones (modified amino acids). Several members of the nuclear receptor family are classified as orphan nuclear receptors because of the lack of known endogenous or synthetic ligands.[3] Estrogen receptor-related receptors (ERRs) are orphan nuclear receptors that are closely related to estrogen receptors (ERα and ERβ). Thus far, three isoforms of ERRs (ERRα, ERRβ, and ERRγ) have been identified.[4] Though they share structural homology and sequence similarity with estrogen receptors, ERRs do not bind with or respond to estrogens, endogenous ER ligands.[5] Among the members of the ERR family, ERRγ is the newest member of the subfamily and is a constitutively active nuclear receptor.[6] ERRγ is highly expressed in a number of adult human and mouse tissues, including tissues in the brain, skeletal muscle, heart, kidney, pancreas, and placenta.[7] ERRγ is also expressed in several human fetal tissues, including tissues in the placenta, brain, heart, skeletal muscle, kidney, and lung. Although little is known about its in vivo functions, the ERRγ-positive status in breast cancer cells may indicate a favorable prognosis in human breast cancer.[8] The discovery of selective small-molecule ligands that would pharmacologically modulate the biological activity of orphan NRs and be utilized as a powerful tool for elucidating the biological roles of these receptors is a breakthrough in the study of biological function of orphan NRs. Because of its potential roles in cancers and metabolic disease, ERRγ has attracted significant attention from the scientific community for the discovery of novel ligands. Recently, 4-hydroxytamoxifen (4-OHT) and diethylstilbestrol (DES), known as antagonist and agonist of ERα, respectively, were reported as inverse agonists of ERRβ and ERRγ. This was confirmed by biochemical and X-ray crystallographic studies.[9] In addition, phenolic acyl hydrazones were reported as functional ERRγ agonists, although in-depth studies of its molecular basis and the observed agonistic response were not carried out.[10] Because of the limited selectivity and specificity of the reported ligands, selective small-molecule ligands for ERRγ have to be obtained for the fundamental understanding of the biological roles of ERRγ.

Under this condition, we aimed to discover novel and selective ERRγ ligands by a rational design along with biomedical analysis and computational docking simulation. In fact, the rational design of small-molecule ligands having the desired selectivity is still far from being achieved because of the complexity of underlying biological phenomena; further, the discovery of small-molecule ligands has been pursued with limited predictability in a low-throughput manner. Therefore, we decided to use an empirical combinatorial approach to significantly enhance the efficiency in synthesis and compensate the limited predictability in rational design.

Results and Discussion

With the goal of discovering a selective ligand of ERRγ, we analyzed the selectivity, affinity, and functional activity

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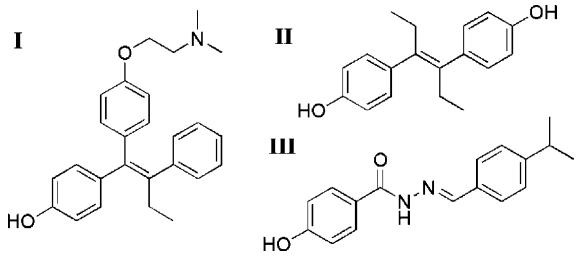


Figure 1. Reported ligands of ERRs. **I**. 4-OHT: Synthetic antagonist of ERα and inverse agonist of ERRγ. **II**. DES: Synthetic agonist of ERα and inverse agonist of ERRγ. **III**. GSK 4716: Synthetic agonist of ERRβ/γ.

of known ligands. When we compared X-ray crystal structures of ERRγ ligand binding domains (LBDs) cocrystallized with GSK4716 and 4-OHT,[11] respectively, we identified the key difference in their interactions in molecular levels at the binding pockets, which might be exploited for the improvement of affinity and selectivity toward ERRγ. As shown in Figure 2a, 4-OHT, a synthetic antagonist of ERα and an inverse agonist of ERRγ, resides mainly in pocket B, and the phenol part of 4-OHT has intact hydrogen bonding with Arg316 and Asp275 at the binding pocket of LBDs. In fact, these two residues serve as gatekeepers by narrowing the hole toward pocket A. In comparison, the phenolic OH group on an aryl ring of GSK4716, a selective agonist of ERRβ/γ, but not a ligand for ERα/β, has a specific interaction with Asp328 as the deep binding pocket (pocket A), and GSK4716 is accommodated throughout pockets A and B. This results from the rearrangement of the gatekeeper residues (Arg316 and Asp275) of LBD (see Figure 2b). When we overlapped the two binding pockets of ERRγ LBDs cocrystallized with 4-OHT and GSK4716, respectively, we could conclude that the selectivity of GSK4716 toward ERRβ/γ over ERα/β was caused by the deep penetration at the binding pocket of the ERRγ LBD. In particular, we found that the phenol part of 4-OHT and isopropylbenzene part of GSK 4716 lie nicely on top of each other in the crystal structures of the 4-OHT- and GSK4716-bound ERRγ LBDs (see Figure 2c). On the basis of this analysis, we could further improve the selectivity of GSK4716 toward ERRγ through the introduction of selectivity elements at pocket B along with the deep penetration at pocket A for specific hydrogen bonding with Asp328, as well as the interaction of a hydrazone moiety with gatekeeper residues. Therefore, the enhanced stabilization of ligands at the ERRγ LBD can ultimately lead to the specific agonist of ERRγ with enhanced affinity over classic ERs. The schematics of the elements used for the selective ERRγ agonist are shown in Figure 2d.

On the basis of this molecular skeleton with an acyl hydrazone moiety derived from computational analysis, we designed and prepared a series of R^1 substituents with hydrazide moieties and R^2 substitutents with benzaldehyde moieties to enhance the binding affinity to ERRγ. Upon condensation by microwave irradiation, the two reactants react to afford the final compounds via the efficient formation of a hydrazone moiety at the linker region.

First, hydrazide building blocks were designed to maximize the efficient hydrogen bondings with Asp328. Therefore, R^1 substituents were incorporated with aryl or heteroaryl moieties containing a hydroxy or amino group (Scheme 1). Hydrazides **A** and **D** were directly borrowed from previously reported compounds.[10] The crystal structure of GSK4716-bound ERRγ LBD reveals that the C(3) atom of GSK4716 hydrazide is adjacent to the amide N−H of the peptide backbone of Asp328 and the ε-amino group of Lys248 with distances of 3.8 Å and 4.7 Å, respectively, suggesting that the introduction of a hydrogen bonding element at the C(3) position of an aryl moiety on the hydrazide building blocks might induce additional hydrogen bonding at the binding pocket and enhance affinity and selectivity to ERRγ. In order to test our hypothesis, we designed and synthesized hydrazide building blocks **B**, **C**, and **E** through the exchange of the C(3) atom with nitrogen to introduce pyridine (**B**, **E**) and pyridone (**C**). The hydrazide building blocks were synthesized with excellent yield and purity through a microwave-assisted nucleophilic acyl substitution reaction of methyl ester with hydrazine (see Supporting Information).

Aldehyde building blocks with R^2 substituents were designed to maximize the selective interaction of small-molecule ligands at pocket B along with the synergistic binding at the deep binding pocket A on the binding site of the ERRγ LBD. As shown in Scheme 2, aldehyde building blocks **1** and **2** were directly borrowed from previously reported building blocks.[10] Aldehydes **3** and **4** with the DES and 4-hydroxytamoxifen moiety, respectively, were designed to introduce tighter binding at pocket B of the ERRγ LBD. In fact, the size of pocket B at the GSK4716-bound ERRγ LBD is much smaller than the size of that at the 4-OHT-bound ERRγ LBD, probably because of the induced fit of the protein. To compensate for this reduced space at the binding pocket with specificity, we designed trimmed moieties of DES and 4-OHT, such as stilbene and its isostere, azobenzene for aldehyde building blocks **5** and **6**, respectively. Aldehyde building blocks **1**, **2**, and **5** are commercially available, but aldehydes **2**, **3**, and **6** were prepared in-house using the synthetic routes shown in Scheme 3. McMurry coupling between **7** and propiophenone produced **8** (E/Z = 3.2:1), and subsequently, $LiAlH_4$ (LAH) reduction, followed by Dess−Martin periodinone (DMP) oxidation, yielded the desired aldehyde building block **3**. Aldehyde **4** was synthesized from **9** through a series of reactions: Mono-protection of **9**, Pd-mediated CO insertion, LAH reduction, DMP oxidation, deprotection, and O-alkylation. Aldehyde **6** was also prepared easily through LAH reduction and DMP oxidation. Finally, we could complete a set of six aldehyde building blocks (see Supporting Information).

For the preliminary evaluation of our designed molecular skeletons, we executed virtual screening by computer-aided docking simulation performed using the Discovery Studio 1.7 program. We utilized previously reported X-ray crystal structures of the GSK4716-bound and 4-OHT-bound ERRγ LBDs (PDB IDs 2GPP and 2GPU, respectively),[11] and the individual small molecules were evaluated for their potential binding to receptor cavities of ERRγ LBDs using the LigandFit module implemented with the Receptor−Ligand Interaction (RLI) protocol (see Supporting Information for the detailed procedure). The dock scores of individual molecules toward two receptor cavities of GSK4716-bound

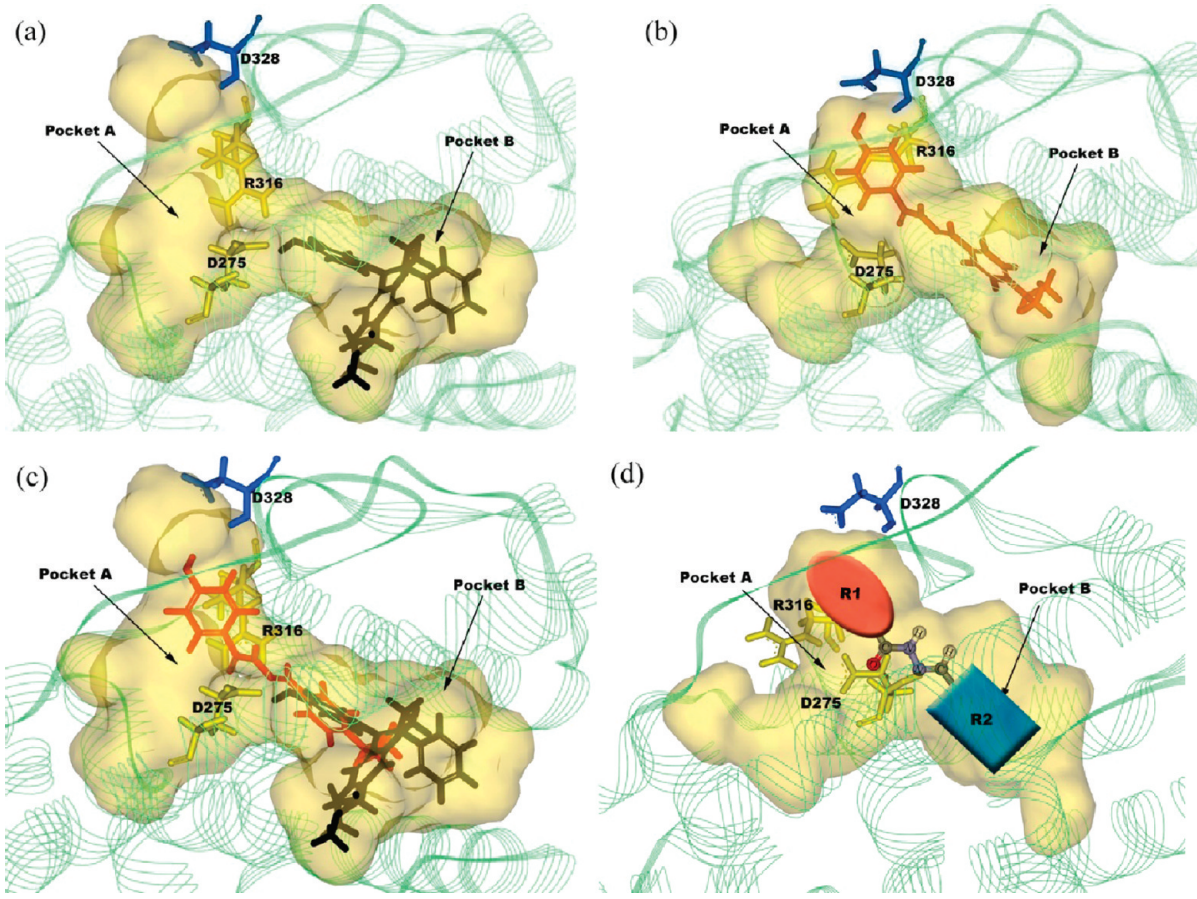
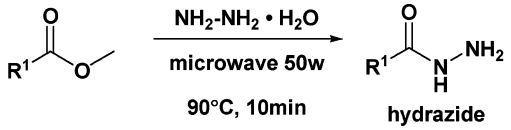


Figure 2. X-ray crystal structures of a ligand-bound ERRγ ligand binding domain (LBD) and schematic illustration of our combinatorial approach for the discovery of selective small-molecule ligands to the ERRγ LBD. (a) 4-Hydroxytamoxifen(4-OHT)-bound LBD of ERRγ (PDB ID 2GPU). (b) GSK4716-bound LBD of ERRγ (PDB ID 2GPP). (c) Overlay of GSK4716 on 4-OHT-bound LBD of ERRγ. (d) Schematic illustration of the designed template used in our combinatorial approach at LBD of ERRγ (PDB ID 2GPP).

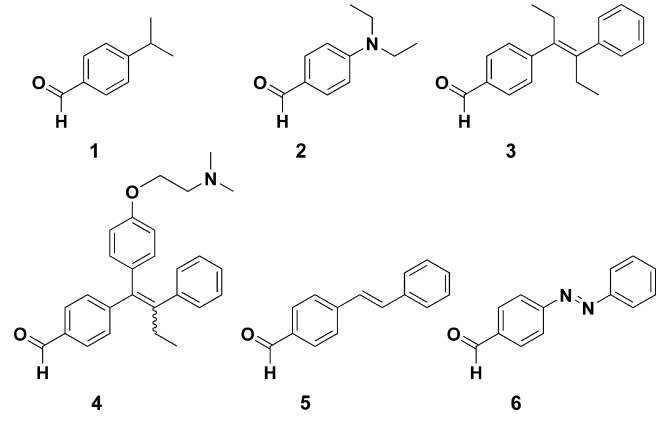
Scheme 1. Preparation of Hydrazide Building Blocks[a,b]



R^1 group	product	yield[a] (%)
4-hydroxyphenyl	A	–[b]
6-hydroxypyridin-3-yl	B	94%
1-methyl-6-oxo-1,6-dihydropyridin-3-yl	C	95%
4-aminophenyl	D	99%
6-aminopyridin-3-yl	E	86%

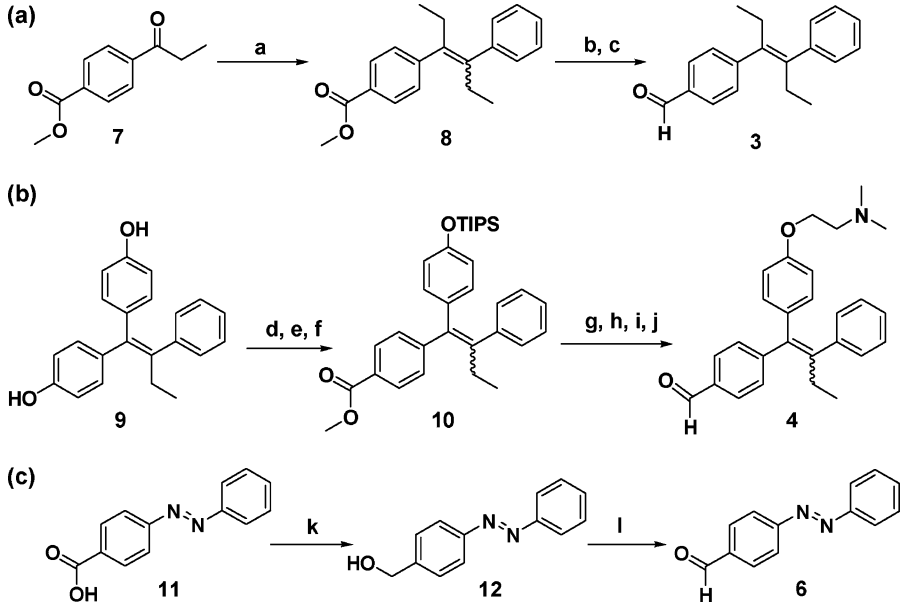
[a] Isolated yield. [b] Commercially available.

Scheme 2. Design of Aldehyde Building Blocks



and 4-OHT-bound ERRγ LBDs are summarized in Tables S1 and S2, Supporting Information. As expected, the ligands with similar molecular skeletons to GSK4716 were fitted nicely to the binding pocket of the ERRγ LBD that is derived from the GSK4716-bound crystal structure (PDB ID 2GPP), whereas the other ligands with bulkier substituents at the R^2 position did not fit the binding pocket, particularly pocket B. In fact, for compounds with aldehydes **5** and **6**, reasonable binding events were observed at the receptor cavity of the GSK4716-bound ERRγ LBD. In the case of the docking study involving the receptor cavity of the 4-OHT-bound ERRγ LBD (PDB ID 2GPU), we did not observe any significant binding events because of the tight arrangement of gatekeeper residues (Arg316 and Asp275) between

Scheme 3. Synthetic Methods for Aldehyde Building Blocks (**3**, **4**, and **6**)[a]



[a] Reagents and conditions: (a) propiophenone, $TiCl_4$, Zn, THF, microwave, 70 °C, 40 min, 65% (*E*/*Z* = 3.2:1); (b) LAH, THF, rt, 2 h, 95%; (c) Dess−Martin periodinane, DCM, rt, 2 h, 95%; (d) TIPSCl, imidazole, DCM, 0 °C → rt, 74%; (e) Tf_2O, 2,6-lutidine, DMAP, DCM, 0 °C → rt, 5 h; (f) CO (5 atm), $Pd(OAc)_2$, dppp, TEA, MeOH/DMF, 70 °C, 20 h, 67%; (g) LAH, THF, rt, 2 h, 97%; (h) Dess−Martin periodinane, DCM, rt, 2 h, 95%; (i) TBAF, THF, rt, 2 h, 92%; (j) 2-chloro-*N*,*N*-dimethylethanamine, Cs_2CO_3, DMF, microwave, 80 °C, 78%; (k) LAH, THF, rt, 2 h, 62%; (l) Dess−Martin periodinane, DCM, rt, 2 h, 77%.

pockets A and B of the LBD. Although we did not achieve the systematic pattern of prediction for the selective binding events on the receptor cavities of ERRγ LBDs, we witnessed slight indication of the potential selectivity for the discovery of novel ligands for ERRγ through the introduction of the bulkier residue at the R^2 position in our modified skeleton.

After the preparation of hydrazide (**A**−**E**) and aldehyde (**1**−**6**) building blocks, we synthesized a series of acyl hydrazone compounds through microwave-assisted solution phase parallel synthesis for the discovery of more potent small-molecule ligands of ERRγ. Scheme 4 shows the structures of the final acyl hydrazones along with their synthetic yields. The microwave-assisted condensation of hydrazide and aldehyde in *n*-BuOH provided excellent yields and purities of acyl hydrazone with a significant enhancement in the reaction rates compared to conventional thermal reactions. The identity of each of the final compounds was confirmed by $^1H/^{13}C$ NMR and mass spectrometry (see Experimental Section and Supporting Information).

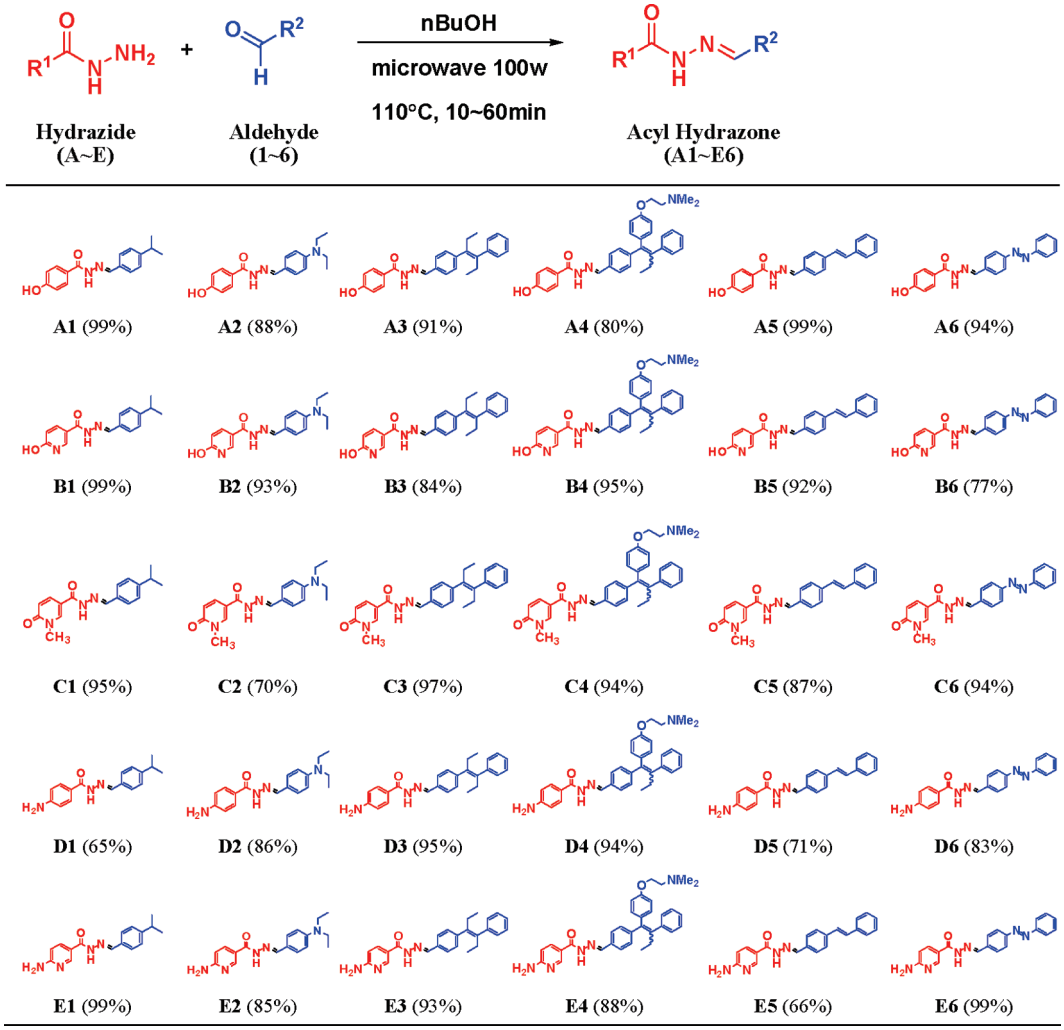
Upon the completion of the full matrix with the 30-member focused library of acyl hydrazones, we pursued the biological evaluation of the resulting compound collection using a cell-based reporter-gene assay to monitor the regulation of the transcriptional activity of ERRγ upon the treatment of small-molecule ligands. A mammalian one-hybrid assay was performed in COS-1 cells using transient transfection with a vector expressing the ligand binding domain (LBD) of ERRγ fused to the Gal4 DNA binding domain (DBD) and a reporter plasmid containing five Gal4 binding sites upstream of the firefly luciferase gene in the pGL2 vector. We used GSK 4716 as a known ERRγ agonist and 4-OHT as a known ERRγ inverse agonist to compare the constitutive activity of unliganded ERRγ. Many compounds of this focused library showed similar activities at the 1 μM concentration to those of GSK4716, probably due to their shared pharmacophore, acyl hydrazone.[10] We were pleasantly surprised to find that **E6** significantly enhanced the transcriptional activity of ERRγ in the cell-based reporter-gene assay system in COS-1 cells (see Figure 3). As we discussed in our previous in silico analysis, **E6** was speculated to be accommodated at the binding cavity of the ERRγ LBD both at pockets A and B through the induced fit, which might lead to its selective agonism on ERRγ but not on ERRα and β (see Figure 4 and Supporting Information).

The excellent potency of **E6** for the transcriptional activity of ERRγ encouraged us to further investigate the biological functions of **E6**, along with its selectivity toward ERRα, β, and γ. For verifying its selectivity toward ERRγ over ERRα and β, the LBD regions of ERRα and β were PCR-amplified in the same plasmid construct (pGL2 vector) used in ERRγ. As shown in Figure 5, **E6** serves as a potent agonist of ERRγ and shows transcriptional activity, even at a 10 nM concentration and the efficacy of **E6** at this concentration was comparable to that of the leading agonist, GSK 4716 at 1 μM concentration. More importantly, **E6** demonstrated the excellent selectivity toward ERRγ without any agonistic effects on ERRα and ERRβ.

Conclusion

With the goal of discovering a selective agonist of ERRγ with enhanced potency, we designed a series of small-molecule ligands derived from a known ERRγ agonist, GSK4716, that can substantially potentiate the transcriptional activity of ERRγ. Individual compounds among a 30-member library of acyl hydrazones were pre-evaluated through *in-silico* docking studies on the receptor cavities of ERRγ LBDs using X-ray crystal structures cocrystallized

Scheme 4. Combinatorial Synthesis of Acyl Hydrazone Using Condensation between a Hydrazide and Aldedyde



with either GSK4716 or 4-OHT. This rational approach to achieve the enhanced potency in ERRγ transcriptional activity with selectivity over ERRα/β enables us to pursue the design and construction of a focused library by carrying out microwave-assisted parallel synthesis with excellent yields and purities. The biological evaluation of focused library members was pursued through the cell-based reporter-gene assay in COS-1 cells, and we identified a more potent ERRγ agonist, **E6**, with excellent selectivity over ERRα/β. Selective ERRγ agonist **E6** discovered in this study is 2 orders of magnitude more potent than the known ERRγ agonist GSK4716. Especially, we would like to emphasize that the systematic combinatorial approach can overcome the limited predictability of in-silico screening with nonflexible docking algorithm. In fact, the new ERRγ agonist **E6** was not predicted as a tight binder toward ERRγ. Therefore, we have successfully demonstrated the inevitability and efficiency of interdisciplinary approach comprised of in silico analysis for enhancing the selective binding at the receptor cavity, combinatorial construction of a focused library by carrying out microwave-assisted solution-phase synthesis, and a cell-based reporter gene assay with a panel of ERRα/

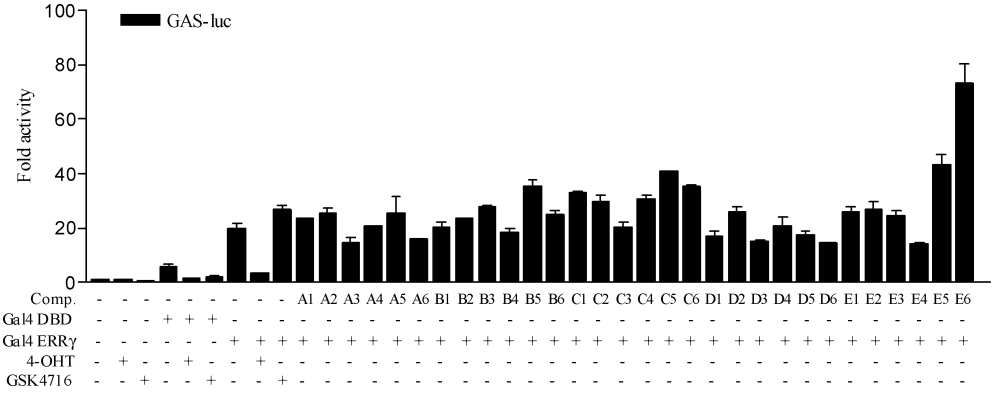


Figure 3. Cell-based reporter-gene assay data for 30 small-molecule ligands.

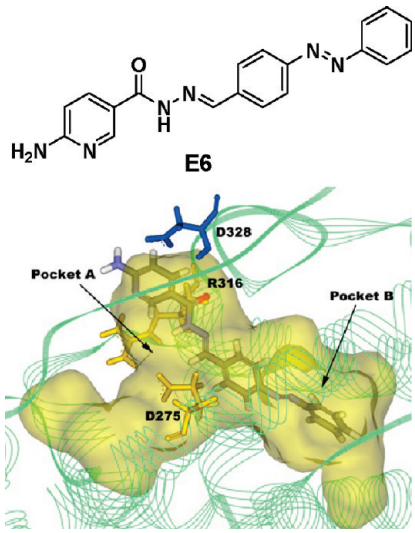


Figure 4. Novel synthetic agonist of ERRγ (**E6**) and the predicted binding mode of **E6** at the binding cavity of ERRγ LBD.

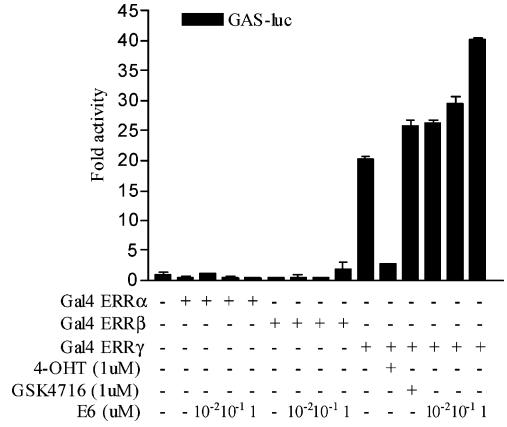


Figure 5. Selectivity of E6 between ERRα, ERRβ, and ERRγ.

β/γ for the discovery of a novel ERRγ agonist. The biological application of the new ERRγ agonist and its mode of action will be reported in due course.

Experimental Section

General Information. All reactions were performed either in oven-dried glassware or microwave vessel under dry argon atmosphere. Toluene and tetrahydrofuran (THF) was dried by distillation from sodium-benzophenone immediately prior to use. Dichloromethane (DCM) was dried by distillation from CaH_2. Other solvent and organic reagent were purchased from commercial venders and used without further purification unless otherwise mentioned. ^1H and ^{13}C NMR spectra were obtained on 300 and 500 MHz FT-NMR spectrophotometer. Chemical shifts were reported in ppm relative to the residual solvent peak for $CDCl_3$ (^1H, 7.24; ^{13}C, 77.23), CD_3OD (^1H, 3.31; ^{13}C, 49.15), $(CD_3)_2CO$ (^1H, 2.05; ^{13}C, 29.92), and $(CD_3)_2SO$ (^1H, 2.50; ^{13}C, 39.51). Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), m (multiplet), dd (doublet of doublet), ddd (doublet of doublet of doublet), dt (doublet of triplet), td (triplet of doublet), br s (broad singlet), etc. Coupling constants were reported in hertz. Routine mass analyses were performed on LC/MS system equipped with a reverse phase column (C-18, 50 × 2.1 mm, 5 μm) and photodiode array detector using electron spray ionization (ESI) or atmospheric pressure chemical ionization (APCI). The high resolution mass spectrometric analyses were conducted at the Mass Spectrometry Laboratory of Seoul National University using mass spectrometer by direct injection for fast atomic bombardment (FAB). Microwave reaction was performed using CEM Discover Benchmate and microwave reaction conditions were denoted in Experimental Section. The products were purified by flash column chromatography on silica gel (230−400 mesh). The eluent used for purification is reported in parentheses. Thin-layer chromatography (TLC) was performed on precoated glass-backed plates (silica gel 60 F_{254} 0.25 mm), and components were visualized by observation under UV light (254 and 365 nm) or by treating the plates with anisaldehyde, $KMnO_4$, phosphomolybdic acid, and vaniline followed by heating. Distilled water was polished by ion exchange and filtration.

Gal4 Fusion Constructs and Transient Transfection Assay. The LBD region of ERRα, β, and γ were PCR amplified using Pfu DNA polymerase and digested with *Eco*RI/*Xho*I and ligated into *Eco*RI/*Sal*I-digested pCMX-Gal4 DBD.[12] COS-1 cells were maintained in DMEM (Hyclone) supplemented with 10% fetal bovine serum (FBS, Hyclone) and antibiotics (Hyclone) in a humidified atmosphere containing 5% CO_2 at 37 °C. The day before transfection, cells were seeded into 24-well plates at a density of 2−8 × 104 cells/well. Transient transfections were performed along with a reporter construct containing five Gal4 binding sites upstream of the firefly luciferase gene in the pGL2 vector (Promega) using SuperFect (Qiagen) for COS-1, according to the manufacturer's instructions. The cells were treated with 4-OHT, GSK4716, or compounds for final 24 h after transfection. Luciferase activity was measured 48 h after transfection and normalized to β-galactosidase activity.

General Procedure for Synthesis of Acyl Hydrazone A1−E6. To a solution of hydrazide of *n*-BuOH (0.1M) was added aldehyde (1.2 equiv.) and the resulting mixture was heated in capped microwave vessel under microwave irradiation (100 W, 100 °C) for 5−60 min. After reaction was complete as monitored by TLC, the crude product was condensed under reduced pressure without aqueous workup procedure and purified by silica-gel flash column chromatography to provide desired products. Some final compounds, such as **B1**, **B2**, **A5−E5**, and **A6−E6**, were purified by crystallization. The residual starting materials and impurities were removed by simple washing with MeOH and DCM.

Characterization of Compound A1: ^1H NMR (300 MHz, CD_3OD) δ 8.30 (s, 1H), 7.85 (d, J = 8.6 Hz, 2H), 7.77 (d, J = 8.0 Hz, 2H) 7.33 (d, J = 8.1 Hz, 2H), 6.90 (d, J = 8.7 Hz, 2H), 3.01−2.92 (m, 1H), 1.29 (d, J = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 162.8, 153.1, 150.2, 133.5, 131.0, 129.0, 128.0, 127.9, 125.1, 116.5, 116.4, 35.5, 24.3; LRMS(ESI$^+$) *m/z* calcd for $C_{17}H_{19}N_2O_2$ $[M + H]^+$ 283.14; found 283.08.

Characterization of Compound A2: ^{1}H NMR (300 MHz, CD_3OD) δ 8.17 (s, 1H), 7.82 (d, J = 8.6 Hz, 2H), 7.65 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 6.73 (d, J = 8.9 Hz, 2H), 3.45 (q, J = 7.0 Hz, 4H), 1.20 (t, J = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 166.6, 162.6, 151.4, 151.1, 130.8, 130.7, 125.3, 122.3, 116.4, 112.5, 45.5, 13.0; LRMS(ESI$^+$) *m/z* calcd for $C_{18}H_{22}N_3O_2$ [M + H]$^+$ 312.17; found 312.13.

Characterization of Compound A3: a mixture of stereoisomers (*E*/*Z* = 85:15); ^{1}H NMR (300 MHz, acetone-d_6) δ 10.83 (s, 0.85 × 1H), 9.06 (s, 0.85 × 1H), 7.93 (d, J = 8.6 Hz, 0.15 × 2H), 7.88 (d, J = 8.6 Hz, 0.85 × 2H), 7.78 (d, J = 7.9 Hz, 0.15 × 2H), 7.44 (d, J = 8.0 Hz, 0.85 × 2H), 7.39 (d, J = 7.7 Hz, 0.15 × 2H), 7.33−7.25 (m, 0.15 × 5H), 7.10−6.91 (m, 9H), 2.65−2.56 (m, 0.85 × 4H), 2.21−2.14 (m, 0.15 × 4H), 0.99−0.93 (m, 0.85 × 6H), 0.80−0.75 (m, 0.15 × 6H); ^{13}C NMR (75 MHz, acetone-d_6) δ 161.6, 146.1, 143.9, 140.9, 139.7, 133.3, 131.1, 130.6, 130.1, 129.6, 129.1, 128.5, 128.0, 127.3, 126.7, 125.9, 116.0, 28.0, 27.7, 13.7, 13.6; LRMS(APCI$^+$) *m/z* calcd for $C_{26}H_{27}N_2O_2$ [M + H]$^+$ 399.21; found 399.10.

Characterization of Compound A4: a mixture of stereoisomers (*E*/*Z* = 46:54); ^{1}H NMR (500 MHz, CD_3OD) δ 8.34 (s, 0.54 × 1H), 8.14 (s, 0.46 × 1H), 7.85 (d, J = 8.5 Hz, 0.54 × 2H), 7.80−7.78 (m, 2H), 7.42 (d, J = 8.0 Hz, 0.46 × 2H), 7.24 (d, J = 8.5 Hz, 0.54 × 2H), 7.25−7.06 (m, 0.54 × 5H, 0.46 × 9H), 6.92 (d, J = 8.5 Hz, 0.46 × 2H), 6.88 (d, J = 8.5 Hz, 0.54 × 2H), 6.85 (d, J = 8.5 Hz, 0.46 × 2H) 6.75 (d, J = 9.0 Hz, 0.54 × 2H), 6.56 (d, J = 9.0 Hz, 0.54 × 2H), 4.09 (t, J = 5.3 Hz, 0.46 × 2H), 3.91 (t, J = 5.5 Hz, 0.54 × 2H), 2.77 (t, J = 5.2 Hz, 0.46 × 2H), 2.67 (t, J = 5.2 Hz, 0.54 × 2H), 2.51−2.43 (m, 2H), 2.34 (s, 0.46 × 6H), 2.27 (s, 0.54 × 6H), 0.93−0.89 (m, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 166.96, 166.85, 163.4, 163.3, 159.2, 158.4, 149.8, 149.7, 147.4, 147.2, 144.4, 143.6, 143.5, 143.5, 139.5, 139.4, 137.0, 136.7, 134.2, 133.2, 133.1, 132.4, 131.9, 131.09, 131.05 130.99, 130.95, 129.2, 129.1, 128.8, 127.9, 127.6, 127.4, 124.48, 124.46, 116.63, 116.58, 115.4, 114.7, 66.6, 66.3, 59.2, 59.1, 45.93, 45.86, 30.2, 30.1, 14.09, 14.05; LRMS(ESI$^+$) *m/z* calcd for $C_{34}H_{36}N_3O_3$ [M + H]$^+$ 534.28; found 534.11.

Characterization of Compound A5: ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.12 (s, 1H), 8.41 (s, 1H), 7.80 (d, J = 8.0 Hz, 2H), 7.69 (br m, 4H), 7.63 (d, J = 7.5 Hz, 2H), 7.40−7.28 (m, 5H), 6.86 (d, J = 8.0 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.7, 160.6, 146.4, 138.5, 136.9, 133.7, 129.6, 129.4, 128.7, 127.83, 127.78, 127.3, 126.8, 126.6, 123.9, 115.0; LRMS(ESI$^+$) *m/z* calcd for $C_{22}H_{19}N_2O_2$ [M + H]$^+$ 343.14; found 343.09.

Characterization of Compound A6: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.83 (br s, 1H), 10.17 (s, 1H), 8.52 (br s, 1H), 7.98−7.91 (m, 6H), 7.85 (d, J = 8.5 Hz, 2H), 7.63−7.58 (m, 3H), 6.89 (d, J = 8.6 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.4, 160.5, 152.4, 152.0, 145.8, 139.2, 138.2, 137.2, 131.9, 129.6, 128.1, 123.2, 122.7, 119.5, 111.0; LRMS(APCI$^+$) *m/z* calcd for $C_{20}H_{17}N_4O_2$ [M + H]$^+$ 345.14; found 345.20.

Characterization of Compound B1: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.93 (br s, 1H), 11.46 (s, 1H), 8.31 (br s, 1H), 8.14 (s, 1H), 7.92 (dd, J = 9.5, 3.0 Hz, 1H), 7.61 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 8.5 Hz, 2H), 6.40 (d, J = 9.5 Hz, 1H), 2.93 (quin, J = 6.9 Hz, 1H), 1.22 (d, J = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.3, 160.3, 146.4, 150.6, 147.1, 139.2, 137.9, 132.0, 127.1, 126.8, 119.4, 111.2, 33.4, 23.7; LRMS(ESI$^+$) *m/z* calcd for $C_{22}H_{19}N_2O_2$ [M + H]$^+$ 343.14; found 343.09.

Characterization of Compound B2: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.03 (br s, 1H), 11.29 (s, 1H), 8.18 (s, 1H), 8.08 (s, 1H), 7.90 (d, J = 9.0 Hz, 1H), 7.48 (d, J = 8.5 Hz, 2H), 6.69 (d, J = 9.0 Hz, 2H), 6.39 (d, J = 9.5 Hz, 1H), 3.38 (q, J = 9.0 Hz, 4H), 1.10 (d, J = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.2, 159.8, 148.8, 148.0, 139.1, 137.5, 128.6, 120.6, 119.2, 111.5, 111.1, 43.7, 12.4; LRMS(APCI$^+$) *m/z* calcd for $C_{17}H_{21}N_4O_2$ [M + H]$^+$ 313.17; found 313.06.

Characterization of Compound B3: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.04 (br s, 1H), 11.53 (s, 1H), 8.21 (s, 1H), 8.10 (s, 1H), 7.89 (dd, J = 9.5, 20 Hz, 1H), 7.40 (d, J = 6.5 Hz, 2H), 7.10−6.94 (m, 7H), 6.39 (d, J = 9.5 Hz, 1H), 2.56−2.52 (m, 4H), 0.94−0.89 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 162.2, 144.6, 142.4, 139.4, 138.1, 131.6, 129.9, 129.4, 127.6, 126.2, 125.8, 119.3, 119.1, 111.1, 26.9, 26.5, 13.2, 13.1; LRMS(APCI$^+$) *m/z* calcd for $C_{25}H_{26}N_3O_2$ [M+H]$^+$: 400.20; Found: 400.08.

Characterization of Compound B4: a mixture of stereoisomers (*E*/*Z* = 46:54); ^{1}H NMR (500 MHz, CD_3OD) δ 8.30 (s, 0.54 × 1H), 8.22 (s, 0.54 × 1H), 8.17 (s, 0.46 × 1H), 8.11 (s, 0.46 × 1H), 8.09 (d, J = 9.5 Hz, 0.54 × 1H), 8.04 (d, J = 9.5 Hz, 0.46 × 1H), 7.82 (d, J = 8.0 Hz, 0.54 × 2H), 7.45 (d, J = 8.0 Hz, 0.46 × 2H), 7.30 (d, J = 8.0 Hz, 0.54 × 2H), 7.19−7.09 (m, 0.54 × 5H, 0.46 × 7H), 6.97 (d, J = 9.0 Hz, 0.46 × 2H), 6.93 (d, J = 8.5 Hz, 0.46 × 2H), 6.78 (d, J = 8.5 Hz, 0.54 × 2H), 6.60 (d, J = 8.5 Hz, 0.54 × 2H), 6.59−6.55 (m, 1H) 4.14 (t, J = 5.5 Hz, 0.46 × 2H), 3.98 (t, J = 5.5 Hz, 0.54 × 2H), 2.83 (t, J = 5.3 Hz, 0.46 × 2H), 2.73 (t, J = 5.2 Hz, 0.54 × 2H), 2.54−2.47 (m, 2H), 2.39 (s, 0.46 × 6H), 2.33 (s, 0.54 × 6H), 0.96−0.92 (m, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.7, 163.6, 163.5, 159.2, 158.4, 150.2, 147.7, 147.5, 144.5, 143.58, 143.56, 143.50, 141.1, 139.6, 139.5, 139.3, 137.0, 136.8, 133.9, 133.5, 133.2, 132.9, 132.5, 131.9, 131.1, 130.96, 130.95, 129.2, 129.1, 128.8, 128.0, 127.6, 127.5, 120.5, 115.5, 114.7, 114.1, 66.5, 66.2, 59.2, 59.1, 49.8, 49.7, 45.9, 45.8, 30.2, 30.1, 14.1, 14.0; LRMS(ESI$^+$) *m/z* calcd for $C_{33}H_{35}N_4O_3$ [M + H]$^+$ 535.27; found 535.17.

Characterization of Compound B5: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.96 (br s, 1H), 11.54 (br s, 1H), 8.33 (br s, 1H), 8.16 (s, 1H), 7.93 (dd, J = 9.7, 2.3 Hz, 1H), 7.72−7.67 (m, 4H), 7.62 (d, J = 7.5 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.35−7.26 (m, 3H), 6.41 (d, J = 9.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.9, 138.5, 136.7, 133.3, 129.39, 129.35, 129.3, 128.4, 127.62, 127.57, 127.57, 127.48, 127.0, 126.6, 126.3, 118.9, 118.8, 111.0; LRMS (APCI$^+$) *m/z* calcd for $C_{21}H_{18}N_3O_2$ [M + H]$^+$ 344.14; found 344.15.

Characterization of Compound B6: ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.15 (br s, 1H), 11.82 (s, 1H), 8.45 (s, 1H), 8.17 (s, 1H), 7.98−7.89 (m, 7H), 7.63−7.57 (m, 3H), 6.43 (d, J = 9.5 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ

162.4, 160.5, 152.4, 152.0, 145.8, 139.2, 138.2, 137.2, 131.9, 129.6, 128.1, 123.2, 122.7, 119.5, 111.0; LRMS(APCI$^+$) *m/z* calcd for $C_{19}H_{16}N_5O_2$ $[M + H]^+$ 346.13; found 346.07.

Characterization of Compound C1: ^{1}H NMR (500 MHz, CD_3OD) δ 8.42 (s, 1H), 8.22 (s, 1H), 7.99 (dd, $J = 9.3$, 1.8 Hz, 1H), 7.70 (d, $J = 7.5$ Hz, 2H), 7.28 (d, $J = 8.0$ Hz, 2H), 6.55 (d, $J = 9.5$ Hz, 1H), 3.61 (s, 3H), 2.90 (quin, $J = 7.0$ Hz, 1H), 1.25 (d, $J = 7.0$ Hz, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.0, 163.3, 153.2, 150.4, 143.8, 139.5, 133.1, 129.0, 127.9, 119.5, 113.9, 38.7, 35.4, 24.2; LRMS(APCI$^+$) *m/z* calcd for $C_{17}H_{20}N_3O_2$ $[M + H]^+$ 298.16; found 298.07.

Characterization of Compound C2: ^{1}H NMR (500 MHz, CD_3OD) δ 8.32 (d, $J = 2.5$ Hz, 1H), 8.07 (s, 1H), 7.94 (dd, $J = 9.5$, 2.5 Hz, 1H), 7.52 (d, $J = 9.0$ Hz, 2H), 6.62 (d, $J = 8.5$ Hz, 2H), 6.49 (d, $J = 9.5$ Hz, 1H), 3.55 (s, 3H), 3.37 (q, $J = 7.2$ Hz, 4H), 1.13 (t, $J = 7.0$ Hz, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.0, 162.9, 151.7, 151.1, 143.5, 139.7, 130.8, 121.8, 119.5, 114.3, 112.3, 45.5, 38.8, 13.1; LRMS(APCI$^+$) *m/z* calcd for $C_{18}H_{23}N_4O_2$ $[M + H]^+$ 327.18; found 327.19.

Characterization of Compound C3: a mixture of stereoisomers (*E*/*Z* = 36: 64); ^{1}H NMR (500 MHz, acetone-d_6) δ 8.43 (br s, 1H), 7.98 (s, 0.64 × 1H), 7.94 (s, 0.36 × 1H), 7.77 (d, $J = 7.0$ Hz, 0.64 × 2H), 7.46−6.97 (m, 0.64 × 8H, 0.36 × 10H), 6.43 (d, $J = 9.5$ Hz, 0.64 × 1H), 6.40 (d, $J = 9.5$ Hz, 0.36 × 1H), 3.60 (s, 0.64 × 3H), 3.57 (s, 0.36 × 3H), 2.63−2.60 (m, 0.36 × 4H), 2.20−2.16 (m, 0.64 × 4H), 0.99−0.95 (m, 0.36 × 6H), 0.80−0.77 (m, 0.64 × 6H); ^{13}C NMR (75 MHz, acetone-d_6) δ 163.0, 145.6, 144.2, 143.4, 141.2, 140.9, 139.8, 134.2, 131.3, 130.8, 130.3, 129.7, 129.2, 128.7, 128.6, 128.32, 128.25, 128.1, 127.5, 127.4, 127.2, 126.8, 119.4, 112.7, 38.0, 37.98, 28.2, 27.9, 27.6, 27.3, 13.7, 13.63, 13.58, 9.9; LRMS(APCI$^+$) *m/z* calcd for $C_{26}H_{28}N_3O_2$ $[M + H]^+$ 414.22; found 414.16.

Characterization of Compound C4: a mixture of stereoisomers (*E*/*Z* = 45: 55); ^{1}H NMR (500 MHz, CD_3OD) δ 8.45 (s, 0.55 × 1H), 8.39 (s, 0.45 × 1H), 8.29 (s, 0.55 × 1H), 8.10 (s, 0.45 × 1H), 8.00 (d, $J = 9.0$ Hz, 0.55 × 1H), 7.95 (d, $J = 9.0$ Hz, 0.45 × 1H), 7.77 (d, $J = 7.5$ Hz, 0.55 × 2H), 7.40 (d, $J = 8.0$ Hz, 0.45 × 2H), 7.26 (d, $J = 8.0$ Hz, 0.55 × 2H), 7.27−7.07 (m, 0.55 × 5H, 0.45 × 7H), 6.94 (d, $J = 8.5$ Hz, 0.45 × 2H), 6.90 (d, $J = 8.5$ Hz, 0.45 × 2H), 6.76 (d, $J = 8.5$ Hz, 0.55 × 2H), 6.58 (d, $J = 9.0$ Hz, 0.55 × 2H), 6.54−6.51 (m, 1H), 4.11 (t, $J = 5.5$ Hz, 0.45 × 2H), 3.93 (t, $J = 5.2$ Hz, 0.55 × 2H), 3.60 (s, 0.55 × 3H), 3.57 (s, 0.45 × 3H), 2.77 (t, $J = 5.5$ Hz, 0.45 × 2H), 2.66 (t, $J = 5.5$ Hz, 0.55 × 2H), 2.52−2.44 (m, 2H), 2.34 (s, 0.45 × 6H), 2.27 (s, 0.55 × 6H), 0.93−0.90 (m, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.05, 165.02, 163.5, 163.4, 159.3, 158.5, 150.3, 150.2, 147.7, 147.5, 144.5, 143.96, 143.89, 143.6, 143.5, 139.68, 139.64, 139.5, 139.4, 137.0, 136.7, 133.9, 133.2, 132.9, 132.5, 131.9, 131.1, 130.97, 130.95, 129.2, 129.1, 128.8, 128.0, 127.6, 127.5, 119.7, 119.6, 115.5, 114.7, 114.04, 114.00, 66.7, 66.5, 59.3, 59.2, 46.02, 45.96, 38.8, 30.2, 30.1, 14.1, 14.0; LRMS (APCI$^+$) *m/z* calcd for $C_{34}H_{38}N_4O_3$ $[M + H]^+$ 550.29; found 550.12.

Characterization of Compound C5: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.64 (s, 1H), 8.48 (s, 1H), 8.36 (s, 1H), 7.93 (dd, $J = 9.5$, 2.0 Hz, 1H), 7.72−7.69 (m, 4H), 7.63 (d, $J = 8.0$ Hz, 2H), 7.41−7.28 (m, 5H), 6.47 (d, $J = 9.5$ Hz, 1H), 3.53 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 161.8, 160.3, 146.8, 142.9, 138.8, 137.8, 136.9, 133.4, 129.6, 128.8, 128.0, 127.8, 127.5, 127.0, 126.7, 118.0, 111.2, 37.5; LRMS(ESI$^+$) *m/z* calcd for $C_{22}H_{20}N_3O_2$ $[M + H]^+$ 358.16; found 358.05.

Characterization of Compound C6: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.82 (s, 1H), 8.51 (s, 1H), 8.44 (s, 1H), 7.99−7.91 (m, 7H), 7.64−7.57 (m, 3H), 6.48 (d, $J = 9.5$ Hz, 1H), 3.53 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 161.8, 160.4, 152.4, 152.0, 145.9, 143.1, 137.8, 137.1, 131.9, 129.6, 128.1, 123.2, 122.7, 118.1, 111.1, 37.5; LRMS(APCI$^-$) *m/z* calcd for $C_{20}H_{16}N_5O_2$ $[M - H]^-$ 358.16; found 358.03.

Characterization of Compound D1: ^{1}H NMR (500 MHz, CD_3OD) δ 8.26 (s, 1H), 7.73−7.70 (m, 4H), 7.28 (d, $J = 8.0$ Hz, 2H), 6.70 (dd, $J = 7.0$, 2.0 Hz, 2H), 2.92 (quin, $J = 7.0$ Hz, 1H), 1.25 (d, $J = 6.5$ Hz, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 167.3, 154.1, 152.9, 149.5, 133.6, 130.7, 129.0, 128.0, 121.3, 114.8, 35.6, 24.4; LRMS(APCI$^+$) *m/z* calcd for $C_{17}H_{20}N_3O$ $[M + H]^+$ 282.16; found 282.08.

Characterization of Compound D2: ^{1}H NMR (500 MHz, CD_3OD) δ 8.14 (s, 1H), 7.70 (d, $J = 8.5$ Hz, 2H), 7.62 (d, $J = 8.5$ Hz, 2H), 6.70 (d, $J = 8.5$ Hz, 4H), 3.43 (q, $J = 7.2$ Hz, 4H), 1.18 (t, $J = 7.0$ Hz, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 167.0, 153.8, 151.0, 150.8, 130.7, 130.5, 122.4, 121.7, 114.9, 112.5, 45.5, 13.1; LRMS(APCI$^+$) *m/z* calcd for $C_{18}H_{23}N_4O$ $[M + H]^+$ 311.19; found 311.08.

Characterization of Compound D3: a mixture of stereoisomers (*E*/*Z* = 59:41); ^{1}H NMR (500 MHz, acetone-d_6) δ 10.75 (s, 0.41 × 1H), 10.64 (s, 0.59 × 1H), 8.47 (s, 0.41 × 1H), 8.28 (s, 0.59 × 1H), 7.81−7.74 (m, 3H), 7.44−7.39 (m, 2H), 7.32−7.26 (m, 2H), 7.09−6.97 (m, 4H), 6.74−6.68 (m, 2H), 5.28−5.23 (m, 2H), 2.64−2.58 (m, 0.59 × 4H), 2.21−2.16 (m, 0.41 × 4H), 0.99−0.94 (m, 0.59 × 6H), 0.80−0.76 (m, 0.41 × 6H); ^{13}C NMR (125 MHz, acetone-d_6) δ 152.9, 145.8, 144.9, 144.0, 143.2, 140.8, 140.5, 139.71, 139.65, 134.5, 133.5, 131.0, 130.6, 130.2, 130.0, 129.6, 129.1, 128.5, 127.8, 127.4, 127.1, 126.7, 122.2, 114.0, 29.3, 29.0, 28.1, 27.8, 13.7, 13.65, 13.59, 13.56; LRMS(APCI$^+$) *m/z* calcd for $C_{26}H_{28}N_3O$ $[M + H]^+$ 398.22; found 398.33.

Characterization of Compound D4: a mixture of stereoisomers (*E*/*Z* = 45:55); ^{1}H NMR (500 MHz, CD_3OD) δ 8.31 (s, 0.55 × 1H), 8.12 (s, 0.45 × 1H), 7.80 (d, $J = 8.0$ Hz, 0.55 × 2H), 7.74 (d, $J = 8.5$ Hz, 0.55 × 2H), 7.69 (d, $J = 8.5$ Hz, 0.45 × 2H), 7.43 (d, $J = 8.0$ Hz, 0.45 × 2H), 7.26 (d, $J = 8.0$ Hz, 0.55 × 2H), 7.17−7.07 (m, 0.55 × 5H, 0.45 × 7H), 6.94 (d, $J = 8.5$ Hz, 0.45 × 2H), 6.89 (d, $J = 8.0$ Hz, 0.45 × 2H), 6.76 (d, $J = 9.0$ Hz, 0.55 × 2H), 6.71 (d, $J = 8.5$ Hz, 0.55 × 2H), 6.68 (d, $J = 8.5$ Hz, 0.45 × 2H), 6.58 (d, $J = 9.0$ Hz, 0.55 × 2H), 4.10 (t, $J = 5.2$ Hz, 0.45 × 2H), 3.93 (t, $J = 5.5$ Hz, 0.55 × 2H), 2.76 (t, $J = 5.2$ Hz, 0.45 × 2H), 2.66 (t, $J = 5.5$ Hz, 0.55 × 2H), 2.52−2.45 (m, 2H), 2.34 (s, 0.46 × 6H), 2.27 (s, 0.54 × 6H), 0.94−0.91 (m, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 167.3, 167.2, 159.3, 158.4, 154.2, 154.1, 149.2, 149.1, 147.3, 147.1, 144.4, 143.64, 143.57, 143.5, 139.58, 139.46, 137.1, 136.8, 134.4, 133.3, 133.2, 132.4, 131.9, 131.1, 130.98, 130.76, 130.69, 129.17, 129.11, 128.7, 127.9, 127.6, 127.4, 121.1, 115.5,

114.79, 114.75, 114.66, 66.7, 66.5, 59.3, 59.2, 46.0, 45.9, 30.2, 30.1, 14.1, 14.0; LRMS(APCI$^+$) *m/z* calcd for $C_{34}H_{37}N_4O_2$ $[M + H]^+$ 533.29; found: 533.11.

Characterization of Compound D5: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.47 (s, 1H), 8.40 (s, 1H), 7.71−7.66 (m, 6H), 7.63 (d, J = 7.5 Hz, 2H), 7.41−7.27 (m, 5H), 6.61 (d, J = 8.5 Hz, 2H), 5.78 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.0, 152.3, 145.5, 138.4, 136.9, 133.9, 129.4, 128.8, 127.9, 127.2, 126.9, 126.6, 119.4, 112.6; LRMS(APCI$^+$) *m/z* calcd for $C_{22}H_{20}N_3O$ $[M + H]^+$ 342.16; found 342.26.

Characterization of Compound D6: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.63 (s, 1H), 8.48 (br s, 1H), 7.98−7.91 (m, 6H), 7.70 (d, J = 8.5 Hz, 2H), 7.63−7.57 (m, 5H), 6.61 (d, J = 8.5 Hz, 2H), 5.83 (s, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 152.4, 152.2, 152.0, 137.7, 131.7, 129.5, 127.8, 123.2, 122.6, 119.3, 112.6; LRMS(ESI$^+$) *m/z* calcd for $C_{20}H_{18}N_5O$ $[M + H]^+$ 344.15; found 344.26.

Characterization of Compound E1: ^{1}H NMR (300 MHz, CD_3OD) δ 8.55 (s, 1H), 8.27 (s, 1H), 7.99 (d, J = 7.5 Hz, 1H), 7.74 (d, J = 7.4 Hz, 2H), 7.30 (d, J = 8.0 Hz, 2H), 6.62 (d, J = 8.8 Hz, 1H), 2.94 (quin, J = 6.9 Hz, 1H), 1.27 (d, J = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.4, 163.2, 153.0, 150.0, 149.5, 138.4, 133.3, 128.9, 127.9, 118.3, 109.2, 35.4, 24.2; LRMS(ESI$^+$) *m/z* calcd for $C_{16}H_{19}N_4O$ $[M + H]^+$ 283.16; found 283.17.

Characterization of Compound E2: ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.19 (s, 1H), 8.51 (s, 1H), 8.22 (s, 1H). 7.86 (d, J = 8.1 Hz, 1H), 7.47 (d, J = 8.1 Hz, 2H), 6.70 (d, J = 8.7 Hz, 2H), 6.52 (s, 2H), 6.46 (d, J = 8.7 Hz, 2H), 3.38 (q, J = 6.9 Hz, 4H), 1.11 (t, J = 6.9 Hz, 6H); ^{13}C NMR (125 MHz, CD_3OD) δ 163.2, 151.5, 151.3, 149.4, 138.4, 130.7, 122.4, 119.1, 112.67, 112.64, 109.4, 45.5, 13.0; LRMS(ESI$^+$) *m/z* calcd for $C_{17}H_{22}N_5O$ $[M + H]^+$ 312.18; found 312.11.

Characterization of Compound E3: a mixture of stereoisomers (*E*/*Z* = 75:25); ^{1}H NMR (500 MHz, acetone-d_6) δ 8.68 (s, 0.25 × 1H), 8.63 (s, 0.75 × 1H), 7.96 (d, J = 6.0, 0.75 × 1H), 7.78 (d, J = 8.0, 0.75 × 1H), 7.44−7.26 (m, 3H), 7.09−6.98 (m, 5H), 6.61 (d, J = 9.0, 0.25 × 1H), 6.58 (d, J = 8.5, 0.75 × 1H), 5.99 (s, 2H), 2.64−2.58 (m, 0.75 × 4H), 2.21−2.16 (m, 0.25 × 4H), 0.99−0.94 (m, 0.75 × 6H), 0.80−0.77 (m, 0.41 × 6H); ^{13}C NMR (125 MHz, acetone-d_6) δ 146.0, 144.0, 143.2, 140.9, 139.7, 139.6, 131.1, 130.7, 130.1, 129.6, 129.1, 128.5, 127.9, 127.4, 127.2, 126.7, 119.2, 107.8, 28.1, 27.8, 13.7, 13.6; LRMS(APCI$^+$) *m/z* calcd for $C_{25}H_{27}N_4O$ $[M + H]^+$ 399.22; found 399.28.

Characterization of Compound E4: a mixture of stereoisomers (*E*/*Z* = 45:55); ^{1}H NMR (500 MHz, CD_3OD) δ 8.56 (s, 0.55 × 1H), 8.51 (s, 0.45 × 1H), 8.32 (s, 0.55 × 1H), 8.13 (s, 0.45 × 1H), 7.99 (dd, J = 9.0, 2.0 Hz, 0.55 × 1H), 7.94 (dd, J = 8.8, 1.7 Hz, 0.45 × 1H), 7.81 (d, J = 8.0 Hz, 0.55 × 2H), 7.44 (d, J = 8.0 Hz, 0.45 × 2H), 7.27 (d, J = 8.0 Hz, 0.55 × 2H), 7.18−7.08 (m, 7H), 6.96 (d, J = 8.5 Hz, 0.45 × 2H), 6.91 (d, J = 8.0 Hz, 0.45 × 2H), 6.78 (d, J = 8.5 Hz, 0.55 × 2H), 6.63−6.58 (m, 2H), 4.14 (t, J = 5.5 Hz, 0.45 × 2H), 3.98 (t, J = 5.3 Hz, 0.55 × 2H), 2.87 (t, J = 5.5 Hz, 0.45 × 2H), 2.77 (t, J = 5.3 Hz, 0.55 × 2H), 2.53−2.46 (m, 2H), 2.42 (s, 0.45 × 6H), 2.35 (s, 0.55 × 6H), 0.94−0.91 (m, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 165.6, 165.5, 163.41, 163.37, 159.2, 158.4, 149.8, 149.73, 149.67, 147.5, 147.3, 144.5, 143.62, 143.61, 143.55, 139.6, 139.4, 138.53, 138.47. 137.2, 136.9, 134.2, 133.2, 133.1, 132.4, 131.9, 131.1, 131.0, 129.2, 129.1, 128.8, 128.0, 127.6, 127.5, 118.4, 115.5, 114.7, 109.4, 66.4, 66.1, 59.2, 59.1, 45.8, 45.7, 30.2, 30.1, 14.03, 13.99; LRMS(APCI$^+$) *m/z* calcd for $C_{33}H_{36}N_5O_2$ $[M + H]^+$ 534.29; found 534.06.

Characterization of Compound E5: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.58 (br s, 1H), 8.55 (d, J = 2.0 Hz, 1H), 8.39 (br s., 1H), 7.89 (dd, J = 8.5, 2.5 Hz, 1H), 7.70−7.68 (m, 4H), 7.63 (d, J = 7.0 Hz, 2H), 7.41−7.28 (m, 5H), 6.64 (s, 2H), 6.48 (d, J = 9.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 138.5, 136.9, 133.7, 129.4, 128.8, 127.9, 127.8, 127.3, 126.9, 126.6, 116.7, 106.9; LRMS(ESI$^+$) *m/z* calcd for $C_{21}H_{19}N_4O$ $[M + H]^+$ 343.16; found 343.11.

Characterization of Compound E6: ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.69 (br s, 1H), 8.58 (d, J = 2.5 Hz, 1H), 8.47 (br s., 1H), 7.98−7.90 (m, 7H), 7.64−7.57 (m, 7H), 6.61 (s, 2H), 6.50 (d, J = 9.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 161.8, 152.3, 152.0, 149.1, 145.1, 137.4, 136.6, 131.7, 129.5, 127.9, 123.1, 122.6, 116.6, 106.9; LRMS(APCI$^+$) *m/z* calcd for $C_{19}H_{17}N_6O$ $[M + H]^+$ 345.15; found 345.00.

Supporting Information Available. General procedure for synthesis of hydrazide **B**−**E**, synthetic procedure of aldehyde buiding blocks **3**, **4**, and **6**, ^{1}H and ^{13}C NMR spectra, structural confirmation of *E*/*Z* isomer of aldehyde **4** (*E*/*Z* mixture) via 1-dimensional ^{1}H NOE experiment, and docking test result. This material is available free of charge via the Internet at http://pubs.acs.org.

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