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Structure−Activity Relationships and Cancer-Cell Selective Toxicity of Novel Inhibitors of Glioma-Associated Oncogene Homologue 1 (Gli1) Mediated Transcription

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We report novel inhibitors of Gli1-mediated transcription as potential anticancer agents. Focused chemical libraries were designed and assessed for inhibition of functional cell-based Gli1-mediated transcription and selective toxicity toward cancer cells. The SAR was revealed, and the selectivity of the lead compounds' inhibition of Gli1-mediated transcription over that of Gli2 was determined. Compound **63** (NMDA298-1), which inhibited Gli1-mediated transcription in C3H10T1/2 cells with an IC_{50} of 6.9 μM, showed 3-fold selectivity for inhibiting transcription mediated by Gli1 over that by Gli2. Cell-viability assays were performed to evaluate the chemical library in a normal cell line and a panel of cancer cell lines with or without up-regulated expression of the *Gli1* gene. These compounds decreased the viability of several cancer cell lines but were less active in the noncancerous BJ-hTERT cells.

Introduction

The Sonic Hedgehog (Shh[a])-Gli signaling pathway regulates the patterning and cellular growth of embryos. Inappropriate activation of this pathway is implicated in diverse cancers.[1−6] Shh signaling is initiated by the binding of Shh to the transmembrane protein Patched (Ptch) and the subsequent release of Smoothened (Smo) from the Ptch−Smo complex. This event facilitates nuclear translocation of the Gli family of zinc finger transcription factors, which direct the transcriptional output of the Shh signal.[7−10] Three Gli transcription factors, Gli1, Gli2, and Gli3, are reported in vertebrates. Gli1 appears to be a transcriptional activator, and Gli2 and Gli3 can act as both activators and repressors of transcription.[11,12] Shh expression is not the only activator of Gli1-mediated transcription; other regulatory mechanisms downstream of Smo also up-regulate Shh signaling.[2,13−16] Elevated expression of *Gli1* and its central role in the formation of several cancers has been well documented,[2,12,17,18] especially in childhood sarcoma.[19] Gli1 and Gli2 are required for the tumorigenicity of human glioma stem cells, but Gli3 has very little or no reported role in tumorogenesis.[12,20] *Gli1*-knockout mice show no obvious phenotypic defects. In contrast, *Gli2*- and *Gli3*-knockout mice show skeletal and neural defects and embryonic or perinatal lethality.[1,17,21,22] In juvenile mice, inhibition of the Shh-Gli pathway upstream by a Smo inhibitor causes severe bone defects.[23] These observations suggest that selective inhibitors of Gli1-mediated transcription, a downstream event of the Shh-pathway, would be useful anticancer drugs[24] tolerated well by pediatric patients who need Hedgehog activity for somatic development and growth.

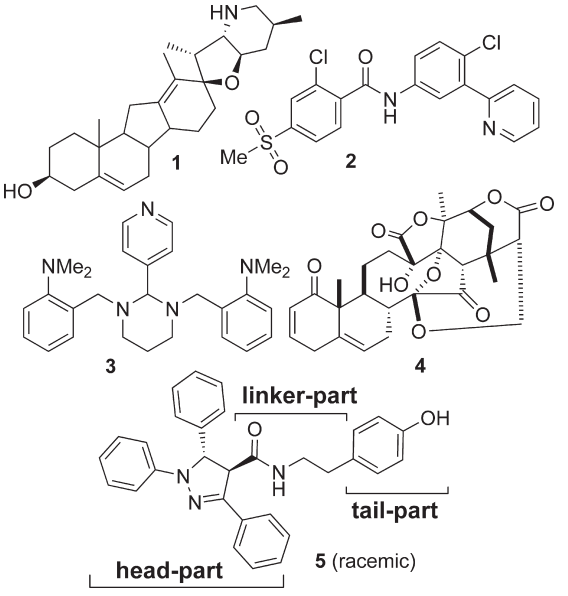

Figure 1. Inhibitors of the Shh-Gli signaling pathway.[24] Compound **5** was chosen as a seed compound for the structure−activity relationship (SAR) investigation. Each partial structure (i.e., the head-, linker-, and tail-parts) discussed in this paper is shown in **5**.

Smo receptor antagonists such as compounds **1** (cyclopamine) and **2** (GDC-0449) (Figure 1), which inhibit Shh signaling at the level of Smo, are in clinical trials.[24] Small-molecule inhibitors of Gli-mediated transcription, compounds **3** (Gant61),[16] **4** (Physalin),[25] and **5** (FN1-8),[26] have also been reported (Figure 1). We designed novel inhibitors of

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[a] Abbreviations: Gli1, glioma-associated oncogene homologue 1; Shh, Sonic Hedgehog; Ptch, Patched; Smo, Smoothened; HBTU, 2-(1*H*-benzo[*d*][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DIPEA, *N,N*-diisopropylethylamine; rt, room temperature.

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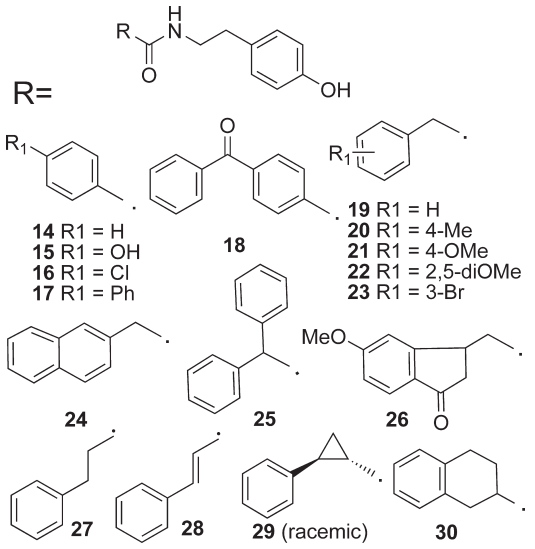
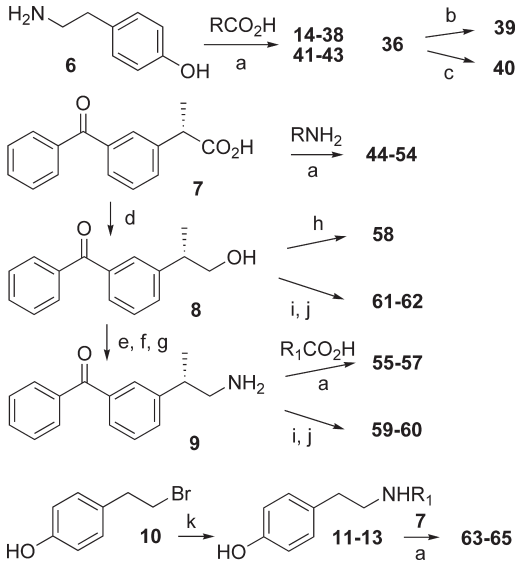


Figure 2. Inactive compounds in the Gli1-mediated transcription assay.

Scheme 1[a]



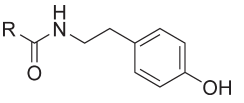
[a] Reagent and conditions: (a) HBTU, DIPEA, DMF, rt; (b) $NaBH_4$, MeOH, 2 h, rt; (c) Pd/H_2, MeOH, 18 h, rt. (d) BH_3−THF, THF, −20 °C to rt; (e) MsCl, Et_3N, CH_2Cl_2, 1 h, 0 °C; (f) NaN_3, DMF, 2 h, 80 °C; (g) PPh_3, NH_4OH, pyridine, rt; (h) R_1-Br, NaH, DMF, rt; (i) 4-nitrophenyl chloroformate, Et_3N, THF, 0 °C to rt; (j) R_1NH_2, Et_3N, THF, 0 °C to rt; (k) R_1NH_2, DIPEA, acetonitrile, 16 h, 60 °C. Structures of compounds **11**−**13** are shown in the Experimental Section, and those of **14**−**65** are shown in Tables 1 and 2 and in Figures 2 and 4.

Gli1-mediated transcription based on **5** by replacing the compound's pyrazoline moiety while retaining a tyramine amide moiety that is an essential pharmacophorefor **5**.[24,26]

Chemistry

The compounds **14**−**38** and **41**−**54** were synthesized by coupling diverse carboxylic acids with appropriate tyramine derivatives (Scheme 1). Compounds **39** and **40** were prepared from **36**. Carboxylic acid **7** was reduced to give alcohol **8**, which on mesylation followed by azide formation and reduction gave the amine **9**. The reverse amides **55**−**57** were synthesized from **9** by reacting with appropriate carboxylic acids. A compound with an ether linker (**58**) was prepared by alkylating **8**. Analogues with a urea linker (**59**−**60**) were synthesized from **9** by first preparing the *p*-nitrophenylcarbamate, which was reacted with corresponding amines. Carbamates (**61**, **62**) were similarly synthesized from alcohol **8**. N-Substituted amides **63**−**65** were synthesized by reacting **10** with appropriate primary amines and then coupling the resulting secondary amines with **7**.

Table 1. Compounds with Different R Groups at the Head-Part of **5**



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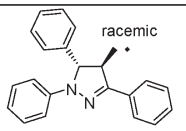
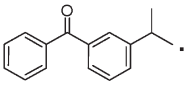
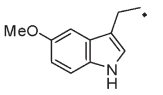
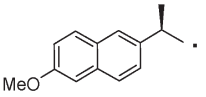
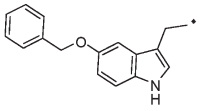
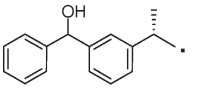
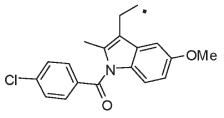
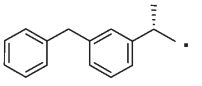
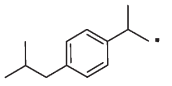
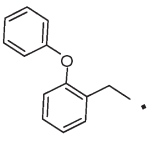
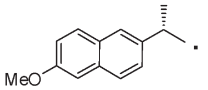
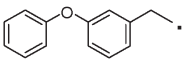
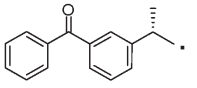
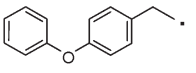
5		**37**	
31		**38**	
32		**39**	
33		**40**	
34		**41**	
35		**42**	
36		**43**	

Results and Discussion

We started our SAR investigation by replacing the headpart of **5** (Figure 1). To assay compounds for selective inhibition of Gli1-mediated transcription, we used C3H10T1/2 mouse embryo fibroblasts with exogenously transfected

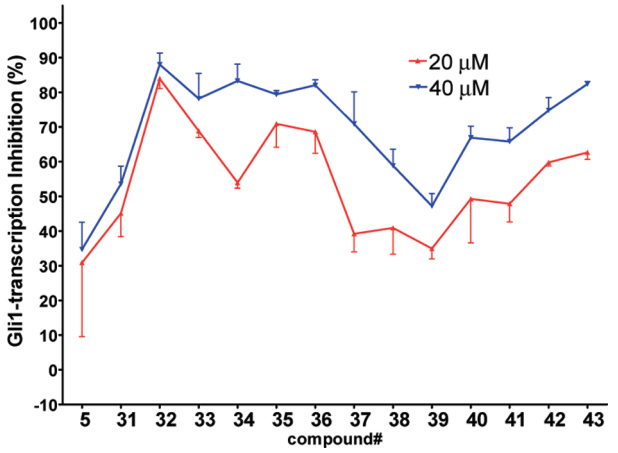
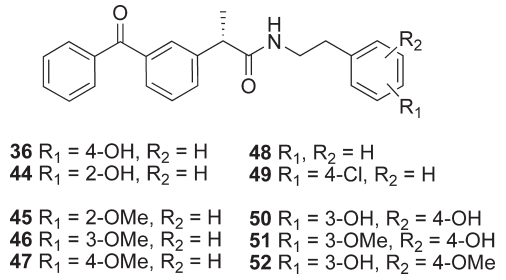


Figure 3. Activity of the head-part library compounds. Percent inhibition of Gli-reporter activity in Gli1-transfected C3H10T1/2 cells 24 h after the addition of 20 μM (red) or 40 μM (blue) of the test compounds (**5**, **31**−**43**). DMSO control = 0%. Error bars represent the SEs of triplicate data.



36 R_1 = 4-OH, R_2 = H
44 R_1 = 2-OH, R_2 = H
48 R_1, R_2 = H
49 R_1 = 4-Cl, R_2 = H
45 R_1 = 2-OMe, R_2 = H
46 R_1 = 3-OMe, R_2 = H
47 R_1 = 4-OMe, R_2 = H
50 R_1 = 3-OH, R_2 = 4-OH
51 R_1 = 3-OMe, R_2 = 4-OH
52 R_1 = 3-OH, R_2 = 4-OMe

Figure 4. SAR library of modified tail-parts of **36**.

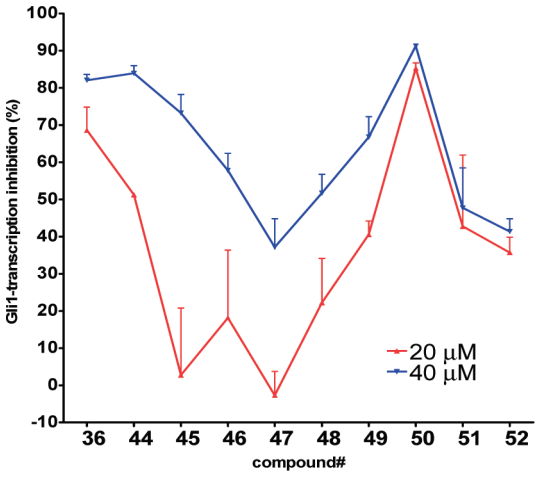
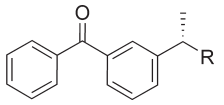
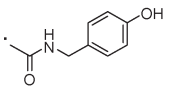
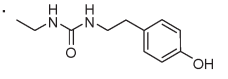
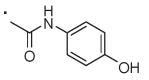
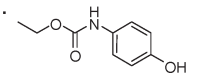
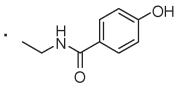
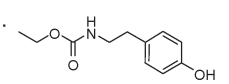
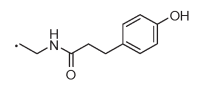
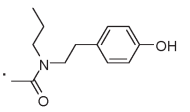
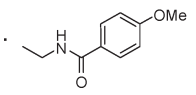
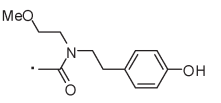
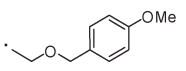
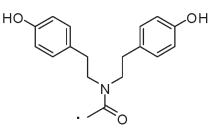
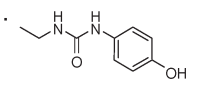


Figure 5. Activity of the tail-part library compounds. Percent inhibition of Gli-reporter activity in Gli1-transfected C3H10T1/2 cells 24 h after addition of 20 μM (red) or 40 μM (blue) of the test compounds (**36**, **44**−**52**). DMSO control = 0%. Error bars represent the SEs of triplicate data.

vectors encoding human Gli1 and a Gli-luciferase reporter vector.[27] Because the Gli-reporter activities in these cells are activated solely by the exogenous Gli1, compounds that down-regulate reporter activity in these cells are believed to target Gli1-mediated transcription but not upstream components such as Smo. Consistently, cyclopamine (**1**), an inhibitor of Smo, is inactive in this assay. Compounds with a small aromatic group as the head-part (**14**−**17**, **19**−**23**) (Figure 2)

Table 2. Analogues for Studying SARs at the Linker-Part of **36**



	R		R
53		**60**	
54		**61**	
55		**62**	
56		**63**	
57		**64**	
58		**65**	
59			

also showed no inhibition of Gli1-mediated transcription (data not shown). We thus increased the size of the aromatic group (**17**, **18**, **24**−**26**) or the distance between the aromatic group and the amide linker (**27**−**30**). The compounds with bulkier aromatic groups and a methylene spacer between the aromatic group and amide (**24**−**26**) showed slight inhibition of Gli1-mediated transcription (data not shown), a finding that suggested the importance of the methylene spacer. Therefore, we next prepared compounds **31**−**36** with the bulkier aromatic group separated from the amide linker by a methylene spacer (Table 1).

Compounds with a bulky group (**32** and **33**) showed better activity than did an analogue with a less bulky group (**31**) (Figure 3). Compound **34**, which has a methyl substitution on the methylene spacer, also showed modest inhibition despite a comparatively smaller aromatic head-part. Compounds that included the methyl group on the methylene spacer and

increased bulk of the aromatic group (**35** and **36**) showed higher activity than **34** at 20 μM and equivalent activity at 40 μM. To evaluate the effect of the chirality on the methylene spacer, we compared the activity of **35** with the activity of its (*R*)-enantiomer **38** and that of **36** with that of the racemic mixture **37**. Compound **38** showed a lower activity than did **35**, and **37** showed a lower activity than did **36**, suggesting a preference for the (S)-enantiomer. The hydroxyl and methylene analogues (**39** and **40**) were less potent than **36**. Replacement of the benzoyl moiety of **36** with a phenoxy group afforded equipotent compounds **42** and **43**, but shifting the phenoxy group to the ortho position (**41**) decreased activity (Figure 3).

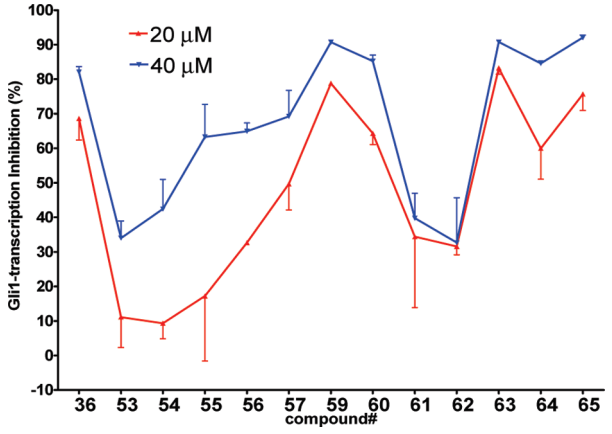


Figure 6. Activity of the linker-part library compounds. Percent inhibition of Gli-reporter activity in Gli1-transfected C3H10T1/2 cells 24 h after addition of 20 μM (red) or 40 μM (blue) of the test compounds (**36**, **53**−**57**, **59**−**65**). DMSO control = 0%. Error bars represent the SEs of triplicate data.

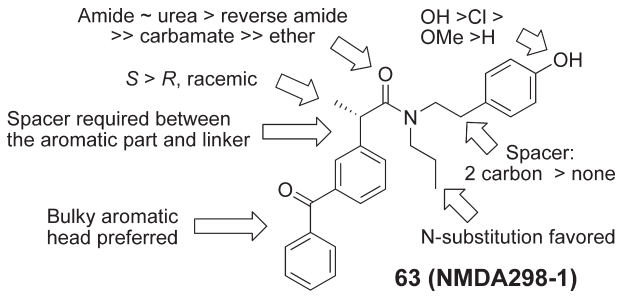


Figure 7. Summary of the structure−activity relationships.

Next, we focused on **36** to investigate the SAR of the tail-part because this compound has high activity and minimal toxicity compared to **32** toward the C3H10T1/2 cells in the reporter assay (data not shown). Compound **7**, in which the whole tail-part was removed, had no activity. Inhibition of Gli1-mediated transcription was slightly decreased at 20 μM when the hydroxyl group was moved to the ortho position (**44**). Replacement of the hydroxyl group with a methoxy group (**45**−**47**) decreased activity. The unsubstituted derivative **48** also showed significantly lower activity than **36**, and the 4-chloro analogue **49** showed slightly lower activity than **36**. The catechol analogue **50** afforded a higher activity than the phenol analogue **36**, but methylation of the catechol (**51** and **52**) reduced the activity by about half. All other substitutions on the benzene ring that were tested, including dichloro, amino, and trifluoromethyl groups or saturation of the benzene ring to a cyclohexyl ring, decreased the activity substantially (data not shown). Overall, the tail-part showed little tolerance for change from phenol (**36**) or catechol (**50**) to any another substituent. (Figures 4 and 5)

Finally, we studied the linker-part by shortening or replacing the amide linker with a substituted amide, reverse amide, ether, urea, or carbamate (Table 2 and Figure 6). Decrease in the length of the linker-part of **36** decreased activity (**53** and **54**). Moving the amidecarbonyl of **53** to the reverse position afforded better activity in **55**; however, extension of the linker length of **55** afforded only a modest increase in the potency of **56**. Despite a decrease in the activity of **47** by methylation of the phenol in the tail-part of **36**, methylation of the reverse amide **55** retained the activity (in **57**). However, the corresponding ether analogue **58** showed almost no activity (data not shown), suggesting the importance of the amide structure in **36** analogues. The potency of compounds with a urea group in the linker-part (**59** and **60**) was comparable to that of **36**, but they later appeared to be toxic to the noncancerous BJ-hTERT cells (Figure 10E). Exchanging the urea with a carbamate substantially decreased the activity (in **61** and **62**). Substitution of the amide nitrogen in **63**−**65** showed activity comparable to or greater than that of **36**.

In summary, the SAR was clearly observed (Figure 7). The scatter plots showed that the library generally followed Lipinski's rule of five and that the activity in Gli1-mediated transcription inhibition does not relate to the calculated

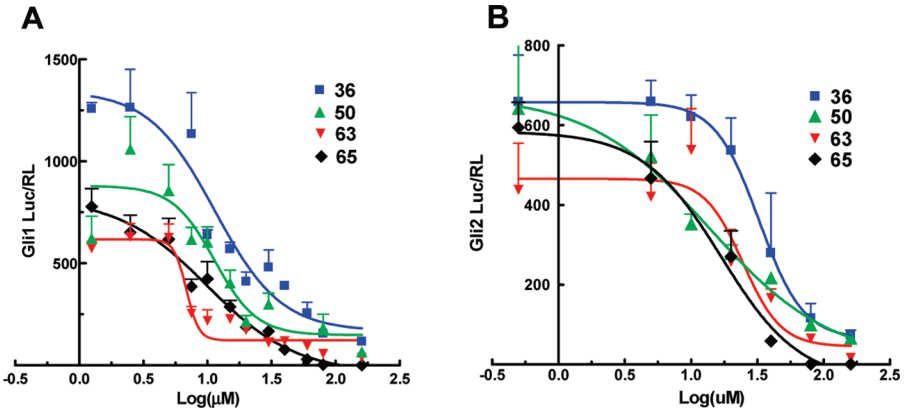


Figure 8. Dose−response curve of Gli-reporter activity in Gli1-transfected (A) or ΔNGli2[29]-transfected (B) C3H10T1/2 cells 24 h after addition of the test compound **36** (blue), **50** (green), **63** (red), **65** (black). Error bars represent the SEs of triplicate data. Dose response curves were fitted using Prism software. Response curve fittings were calculated by Prism software (GraphPad). IC_{50} in these curves is summarized in Table 3.

values of log *P* or total polar surface area for each compound (Supporting Information Figure 1). These findings suggest that the inhibition of Gli1-mediated transcription is not due to hydrophobic aggregation by the compound. To exclude the possibility that these compounds are inhibitors of transcription/translation of the exogenously transfected *Gli1* in the assayed C3H10T1/2 cells, we determined the Gli1 protein levels by Western blot analysis. The *Gli1*-transfected C3H10T1/2 cells have been confirmed to overexpress the *exogenous* Gli1 protein, and it is not reduced by **36** or **63** (Supporting Information Figure 5). This finding suggests that they do not affect the protein expression from the exogenous Gli1 vector or the transgenic promoter. We have also tested the possibility that these compounds are inhibitors of assay reporter.[28] Instead of adding compounds to the cells, they were added to the C3H10T1/2 lysate in which luciferase is highly expressed by the exogenous *Gli1*/*Gli-Luc* reporter. The luciferase activity in these samples was not changed by **36** or **63** (Supporting Information Figure 6), confirming that they are not inhibitors of luciferase reporter.

Table 3. IC_{50} (μM) and 95% Confidence Intervals (in Parentheses) of Selected Compounds for Inhibiting Transcriptional Activation by Exogenous Human Gli1 or ΔNGli2[29] in C3H10T1/2 Cells 24 h after Addition of the Test Compound (Figure 8)

	hGli1	ΔNGli2
36	11.4 (7.5−17.5)	35.2 (17.8−59.9)
50	11.4 (7.9−16.3)	29.4 (2.9−66.6)
63	6.9 (6.1−7.8)	23.9 (12.3−49.0)
65	9.4 (6.40−15.4)	20.5 (9.2−30.9)

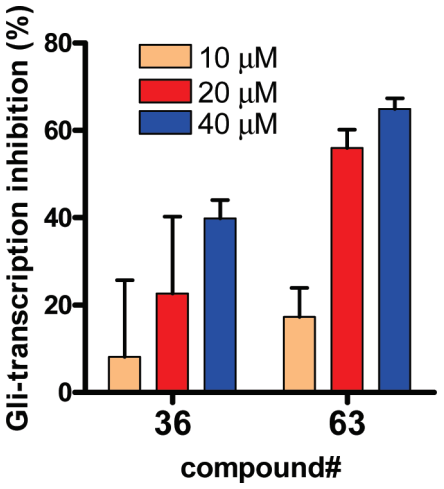


Figure 9. Percent inhibition of Gli-reporter activity in Rh30 cells 24 h after the addition of 10 μM (peach), 20 μM (red), or 40 μM (blue) of the test compound **36** or **63**. DMSO control = 0%. Error bars represent the SEs of triplicate data.

Selected compounds were evaluated for their selectivity in inhibiting the Gli1-mediated transcription over Gli2-mediated transcription by detemining their IC_{50} values (Figure 8 and Table 3). The compounds showed 3−4 times weaker inhibition of Gli2-mediated transcription than that of Gli1 (Table 3). Compound **65** was least selective for Gli1. We also tested the stability of compounds **36**, **50**, **63**, and **65** in the C3H10T1/2 cell cultures. Catechol derivative **50** showed significant degradation, while phenol derivatives **36**, **63**, and **65** were stable for 24 h (Supporting Information Figure 4)

To prove that inhibitors of transcription in C3H10T1/2 cells with exogenously overexpressed *Gli1* are also active in cells that endogenously overexpressed *Gli1*, we assayed compounds **36** and **63** for inhibition of Gli-reporter activity in Rh30, a rhabdomyosarcoma cell line that overexpresses *Gli1* endogenously,[3] without contransfection of exogenous *Gli1*. This assay confirmed that **36** and **63** inhibit transcription by the endogenous Gli1 in this cell line (Figure 9); thus, these compounds are expected to suppress the growth of cancer

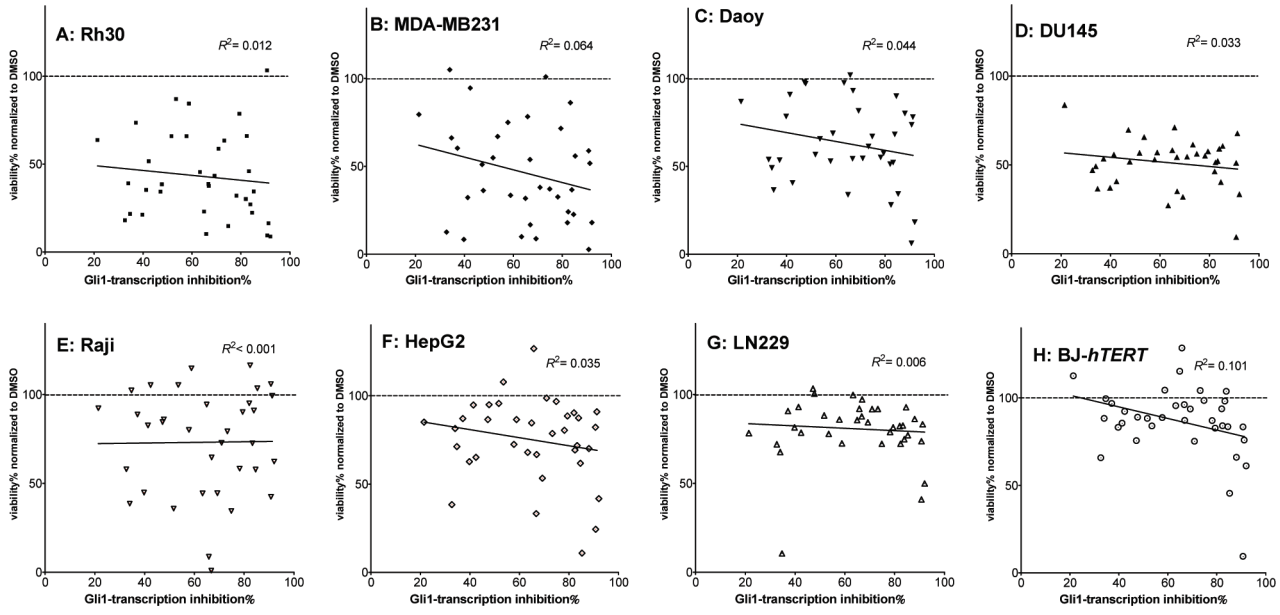


Figure 10. Scatter plots of the inhibition % of Gli1-mediated transcription in the C3H10/T1/2 cells 24 h after treatment with 40 μM of each compound vs the cell viability % of each cell line 72 h after treatment in an Alamar Blue assay. Each point in the plots represents a single compound (**31-65**). Each plot represents each single compound shown in Figures 3, 5, and 6. Both data sets were normalized to those of DMSO as 0% transcription inhibition and 100% cell viability: (A) Rh30, (B) MDA-MB231, (C) Daoy, (D) DU145, (E) Raji, (F) HepG2, (G) LN229, and (H) BJ-hTERT. Linear regressions were calculated by Prism software (GraphPad). Data from individual compounds are shown in the Supporting Information Figure 2.

cells in which growth control is *Gli1*-dependent, such as meduloblastoma progenitor cells.[17]

To determine whether inhibitors of Gli1-mediated transcription inhibit the growth of cancer cells, we tested their effect on the viability of several cancer cell lines. Cell viability was reduced in Rh30 (rhabdomyosarcoma, Figure 10A), MDA-MB231 (breast cancer, Figure 10B), Daoy (medulloblastoma, Figure 10C), DU145 (prostate cancer, Figure 10D), and Raji (Burkitt's lymphoma, Figure 10E) but not in HepG2 (hepatocellular carcinoma, Figure 10F), LN229 (glioma, Figure 10G), and hTERT-immortalized BJ (noncancerous human skin fibroblasts,[30] Figure 10H). This finding suggests that inhibitors of Gli1-mediated transcription could suppress the growth of cancer cells. However, it is difficult to observe the simple relationship between reduction of cell viability and inhibition of Gli1-mediated transcription, likely because of a nonspecific effect of some of the compounds.

It is interesting to see if the growth inhibition effect correlates to the level of Gli1 protein in these cell lines. However, in our hand, the Western blot analysis reliably detects *exo*genous Gli1 protein expression in the C3H10T1/2 cells (Supporting Information Figure 5) but not *endo*genous Gli1 protein in these cell lines for the viability study, with the only exception being Rh30 (data not shown). Expression of *Gli1* mRNA has been previously shown in Rh30,[3] MDA-MB231,[31] Daoy,[32,33] and DU145[34] cell lines. However, it is well-known that Gli1 protein is down-regulated post-translationally[32,35] even when *Gli1* mRNA is expressed,[36] thus, the level of Gli1 protein does not linearly correlate to that of *Gli1* mRNA. A statistical approach with a simple two-category analysis (e.g., *Gli1* mRNA-overexpression or nonoverexpression) suggests that the Gli1-mediated transcription inhibitors decrease the viability of Rh30, MDA-MB231, Daoy, and DU145 cell lines by 0.19% more than that of the other four cell lines, per 1% increase in the inhibition (see Supporting Information for detail).

Conclusion

In this study, we report the discovery of novel inhibitors of Gli1-mediated transcription that are potential anticancer agents. The amide analogue **36** and substituted amide **63** inhibited Gli1-mediated transcription without showing toxicity against a normal cell line, down-regulated endogenous Gli-mediated transcription in Rh30 cells, and demonstrated inhibition selectivity of Gli1-mediated transcription that is approximately 3 times greater than that of Gli2-mediated transcription. Cell viability is reduced by analogues of these compounds in cancer cells. These compounds might be the basis for designing potential lead compounds that function as anticancer agents by selectively inhibiting Gli1-mediated transcription.

Experimental Section

General Procedure. Compounds **14**−**38** and **41**−**56** were synthesized as follows: A mixture of the appropriate carboxylic acid (1 equiv), HBTU (2.5 equiv), and DIPEA (3 equiv) in DMF (0.3 mL) was allowed to stand for 30 min at rt. Appropriate amine (3.0 equiv) was then added to the mixture and stirred for 24 h at rt. Water was added to the reaction mixture and extracted with ethyl acetate followed by successive washings with water and brine. The organic layer was dried over anhydrous sodium sulfate. The solvent was removed in vacuo to give the residue, which was chromatographed over silica gel (Biotage SP4, 12+S column, eluting with hexane/ethyl acetate gradient 10−80%) to give the desired compound.

The purity of all the compounds was determined by HPLC on a Waters Alliance HT LC−MS system (Waters 2795 separation module linked to a Waters 2996 photodiode array detector) using a Waters XBridge C18, 3.5 μm (4.6 mm × 50 mm) column by running a 0−95% gradient for Water (+0.05% TFA)/MeOH (Supporting Information Figure 3). All the compounds showed ≥95% purity, unless otherwise mentioned.

***N*-(4-Hydroxyphenethyl)benzamide (14).** ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ 7.72 (m, 2H), 7.49 (m, 1H), 7.41 (t, 2H, J = 7.4 Hz), 7.08 (d, 2H, J = 8.5 Hz), 6.79 (d, 2H, J = 8.5 Hz), 3.61 (t, 2H, J = 7.2 Hz), 2.84 (t, 2H, J = 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{15}H_{16}NO_2$ 242.1181, found 242.1175.

4-Hydroxy-*N*-(4-hydroxyphenethyl)benzamide (15). ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ 7.57 (d, 2H, J = 8.7 Hz), 7.07 (d, 2H, J = 8.4 Hz), 6.80 (m, 4H), 3.59 (t, 2H, J = 7.0 Hz), 2.82 (t, 2H, J = 7.0 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{15}H_{16}NO_3$ 258.1130, found 258.1125. HPLC purity 93%.

4-Chloro-*N*-(4-hydroxyphenethyl)benzamide (16). ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ 7.70 (d, 2H, J = 8.3 Hz), 7.40 (d, 2H, J = 8.3 Hz), 7.07 (d, 2H, J = 8.2 Hz), 6.78 (d, 2H, J = 8.1 Hz), 3.58 (t, 2H, J = 7.3 Hz), 2.83 (t, 2H, J = 7.3 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{15}H_{15}NO_2Cl$ 276.0791, found 276.0781.

***N*-(4-Hydroxyphenethyl)biphenyl-4-carboxamide (17).** ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ 7.83 (d, 2H, J = 8.2 Hz), 7.64 (dd, 4H, J = 7.7, 16.9 Hz), 7.47 (t, 2H, J = 7.5 Hz), 7.39 (t, 1H, J = 7.4 Hz), 7.11 (d, 2H, J = 7.8 hz), 6.79 (d, 2H, J = 7.6 Hz), 3.62 (t, 2H, J = 6.9 hz), 2.87 (t, 2H, J = 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{21}H_{20}NO_2$ 318.1494, found 318.1491.

4-Benzoyl-*N*-(4-hydroxyphenethyl)benzamide (18). ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ 7.83 (m, 6H), 7.65 (t, 1H, J = 7.4 Hz), 7.52 (t, 2H, J = 7.6 Hz), 7.10 (d, 2H, J = 8.5 Hz), 6.83−6.76 (m, 2H), 3.63 (t, 2H, J = 7.2 Hz), 2.87 (t, 2H, J = 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{22}H_{20}NO_3$ 346.1443, found 346.1436.

***N*-(4-Hydroxyphenethyl)-2-phenylacetamide (19).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (m, 3H), 7.17 (d, 2H, J = 7.2 Hz), 6.86 (d, 2H, J = 8.4 Hz), 6.71 (d, 2H, J = 8.3 Hz), 5.86 (s, 1H), 3.50 (s, 2H), 3.39 (t, 2H, J = 6.7 Hz), 2.63 (t, 2H, J = 6.8 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{16}H_{18}NO_2$ 256.1338, found 256.1335.

***N*-(4-Hydroxyphenethyl)-2-*p*-tolylacetamide (20).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.12 (d, 2H, J = 7.7 Hz), 7.03 (d, 2H, J = 8.0 Hz), 6.86 (d, 2H, J = 8.5 Hz), 6.72 (m, 2H), 5.46 (s, 1H), 3.50 (s, 2H), 3.42 (q, 2H, J = 6.8 Hz), 2.63 (t, 2H, J = 6.9 Hz), 2.34 (s, 3H). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{17}H_{20}NO_2$ 270.1494, found 270.1483.

***N*-(4-Hydroxyphenethyl)-2-(4-methoxyphenyl)acetamide (21).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.05 (d, 2H, J = 6.8 Hz), 6.85 (t, 4H, J = 7.2 Hz), 6.72 (d, 2H, J = 6.5 Hz), 5.45 (s, 1H), 3.80 (s, 3H), 3.47 (s, 2H), 3.42 (dd, 2H, J = 6.4, 13.0 Hz), 2.63 (t, 2H, J = 6.7 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{17}H_{20}NO_3$ 286.1443, found 286.1436.

2-(2,5-Dimethoxyphenyl)-*N*-(4-hydroxyphenethyl)acetamide (22). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.87 (d, 2H, J = 7.4 Hz), 6.78 (d, 3H, J = 14.2 Hz), 6.70 (d, 2H, J = 7.4 Hz), 5.84 (s, 1H), 3.77 (s, 3H), 3.68 (s, 7H), 3.38 (m, 2H), 2.61 (t, 2H, J = 6.8 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{18}H_{22}NO_4$ 316.1549, found 316.1545.

2-(3-Bromophenyl)-*N*-(4-hydroxyphenethyl)acetamide (23). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (d, 1H, J = 7.9 Hz), 7.35 (d, 1H, J = 1.7 Hz), 7.19 (t, 1H, J = 7.8 Hz), 7.11 (d, 1H, J = 7.7 Hz), 6.90 (d, 2H, J = 8.5 Hz), 6.77−6.70 (m, 2H), 5.51 (s, 1H), 5.35 (s, 1H), 3.48 (s, 2H), 3.45 (dd, 2H, J = 6.7, 12.8 Hz), 2.67 (t, 2H, J = 6.7 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{16}H_{16}BrNO_2Na$ 356.0262, found 356.0255.

***N*-(4-Hydroxyphenethyl)-2-(naphthalen-2-yl)acetamide (24).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (dt, 3H, J = 7.2, 16.9 Hz), 7.61 (s, 1H), 7.50 (m, 2H), 7.26 (m, 1H), 6.77 (d, 2H, J = 8.4 Hz), 6.54 (dd, 2H, J = 2.4, 8.9 Hz), 5.41 (s, 1H), 3.69 (s, 2H), 3.41 (q, 2H, J = 6.6 Hz), 2.60 (t, 2H, J = 6.8 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{20}H_{20}NO_2$ 306.1494, found 306.1486.

***N*-(4-Hydroxyphenethyl)-2,2-diphenylacetamide (25).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (m, 6H), 7.15 (d, 4H, J = 6.7 Hz), 6.85 (d, 2H, J = 8.3 Hz), 6.68 (d, 2H, J = 8.3 Hz), 5.82 (s, 1H), 4.87 (s, 1H), 3.48 (t, 2H, J = 6.8 Hz), 2.66 (t, 3H, J = 6.8 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{22}H_{22}NO_2$ 332.1651, found 332.1642.

***N*-(4-Hydroxyphenethyl)-2-(6-methoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetamide (26).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, 1H, J = 8.5 Hz), 7.01 (d, 2H, J = 8.3 Hz), 6.93 (dd, 2H, J = 3.8, 12.3 Hz), 6.77 (d, 2H, J = 8.4 Hz), 5.32 (s, 1H), 3.88 (s, 3H), 3.80 (dd, 1H, J = 7.6, 14.7 Hz), 3.54 (m, 1H), 3.41 (m, 2H), 2.87 (dd, 1H, J = 7.3, 18.7 Hz), 2.73 (m, 2H), 2.63 (dd, 1H, J = 4.8, 14.4 Hz), 2.27 (dd, 1H, J = 9.8, 13.9 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{20}H_{22}NO_4$ 340.1549, found 340.1533.

***N*-(4-Hydroxyphenethyl)-3-phenylpropanamide (27).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (m, 2H), 7.19 (dd, 3H, J = 6.5, 13.3 Hz), 6.94 (d, 2H, J = 8.3 Hz), 6.75 (d, 2H, J = 8.4 hz), 5.32 (m, 1H), 3.43 (dd, 2H, J = 6.6, 12.9 Hz), 2.94 (t, 2H, J = 7.6 Hz), 2.65 (t, 2H, J = 6.8 Hz), 2.42 (t, 2H, J = 7.6 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{17}H_{20}NO_2$ 270.1494, found 270.1504.

***N*-(4-Hydroxyphenethyl)cinnamamide (28).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, 1H, J = 15.6 Hz), 7.48 (s, 2H), 7.36 (m, 3H), 7.09 (d, 2H, J = 8.5 Hz), 6.80 (d, 2H, J = 8.5 Hz), 6.31 (d, 1H, J = 15.6 Hz), 5.58 (m, 1H), 3.62 (d, 2H, J = 6.1 Hz), 2.82 (t, 2H, J = 6.9 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{17}H_{18}NO_2$ 268.1338, found 268.1327. HPLC purity 92%.

(1*SR*,2*SR*)-*N*-(4-Hydroxyphenethyl)-2-phenylcyclopropanecarboxamide (29). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (t, 2H, J = 7.6 Hz), 7.18 (t, 1H, J = 7.3 Hz), 7.05 (m, 4H), 6.78 (d, 2H, J = 8.3 Hz), 5.70 (s, 1H), 3.51 (m, 2H), 2.75 (t, 2H, J = 7.0 Hz), 2.49 (m, 1H), 1.62 (m, 1H), 1.52 (m, 1H), 1.23 (m, 2H). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{18}H_{20}NO_2$ 282.1494, found 282.1491.

***N*-(4-Hydroxyphenethyl)-1,2,3,4-tetrahydronaphthalene-2-carboxamide (30).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.06 (m, 6H), 6.79 (d, 2H, J = 8.5 Hz), 5.64 (t, 1H, J = 5.1 Hz), 3.52 (dd, 2H, J = 6.9, 14.3 Hz), 2.85 (m, 6H), 2.46 (m, 1H), 2.03 (d, 1H, J = 10.2 Hz), 1.86 (m, 1H). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{19}H_{22}NO_2$ 296.1651, found 296.1641.

***N*-(4-Hydroxyphenethyl)-2-(5-methoxy-1*H*-indol-3-yl)acetamide (31).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 7.27 (d, 1H, J = 8.7 Hz), 6.89 (m, 3H), 6.62 (dd, 4H, J = 8.5, 22.2 Hz), 5.88 (s, 1H), 3.64 (s, 2H), 3.35 (t, 2H, J = 5.7 Hz), 2.53 (t, 2H, J = 6.7 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{19}H_{21}N_2O_3$ 325.1552, found 325.1545.

2-(5-(Benzyloxy)-1*H*-indol-3-yl)-*N*-(4-hydroxyphenethyl)acetamide (32). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, 1H, J = 7.5 Hz), 7.38 (t, 1H, J = 7.5 Hz), 7.31 (dd, 1H, J = 4.5, 8.0 Hz), 6.99 (dd, 1H, J = 9.1, 11.5 Hz), 6.64 (d, 1H, J = 8.4 Hz), 6.55 (d, 1H, J = 8.3 Hz), 5.08 (s, 1H), 3.64 (s, 0H), 3.33 (m, 1H), 2.52 (t, 1H, J = 6.6 Hz). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{25}H_{25}N_2O_3$ 401.1865, found 401.1855.

2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)-*N*-(4-hydroxyphenethyl)acetamide (33). ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ 7.64 (d, 2H, J = 8.3 Hz), 7.52 (d, 2H, J = 8.4 Hz), 6.91 (dd, 2H, J = 5.7, 8.1 Hz), 6.80 (d, 2H, J = 8.4 Hz), 6.72 (dd, 1H, J = 2.5, 9.0 Hz), 6.62 (d, 2H, J = 8.4 Hz), 6.29 (s, 1H), 3.84 (s, 3H), 3.58 (s, 2H), 3.40 (dd, 2H, J = 6.6, 12.7 Hz), 2.62 (t, 2H, J = 6.8 Hz), 2.28 (s, 3H). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{27}H_{26}N_2O_4Cl$ 477.1581, found 477.1571.

***N*-(4-Hydroxyphenethyl)-2-(4-isobutylphenyl)propanamide (34).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10 (m, 4H), 6.83 (d, 2H, J = 8.5 Hz), 6.72 (d, 2H, J = 8.6 Hz), 5.44 (t, 1H, J = 5.4 Hz), 3.43 (ddq, 3H, J = 7.0, 13.3, 26.0 Hz), 2.61 (m, 2H), 2.46 (d, 2H, J = 7.2 Hz), 1.85 (d p, 1H, J = 6.8, 13.6 Hz), 1.49 (d, 3H, J = 7.2 Hz), 0.91 (d, 6H, J = 6.6 Hz). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{21}H_{28}NO_2$ 326.2120, found 326.2109.

(*S*)-*N*-(4-Hydroxyphenethyl)-2-(6-methoxynaphthalen-2-yl)propanamide (35). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (t, 2H, J = 7.8 Hz), 7.56 (s, 1H), 7.27 (d, 1H, J = 8.4 Hz), 7.14 (m, 2H), 6.74 (d, 2H, J = 8.3 Hz), 6.59 (d, 2H, J = 8.4 Hz), 5.47 (s, 1H), 3.91 (s, 3H), 3.64 (dd, 1H, J = 6.3, 13.4 Hz), 3.38 (ddd, 2H, J = 6.4, 16.3, 19.2 Hz), 2.57 (t, 2H, J = 6.5 Hz), 1.57 (d, 3H, J = 7.1 Hz). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{22}H_{24}NO_3$ 350.1756, found 350.1754.

(*S*)-2-(3-Benzoylphenyl)-*N*-(4-hydroxyphenethyl)propanamide (36). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (m, 2H), 7.66 (m, 2H), 7.59 (m, 1H), 7.46 (m, 4H), 6.81 (d, 2H, J = 8.5 Hz), 6.68 (m, 2H), 5.54 (t, 1H, J = 5.6 Hz), 3.56 (q, 1H, J = 7.1 Hz), 3.41 (m, 2H), 2.62 (t, 2H, J = 6.8 Hz), 1.51 (d, 3H, J = 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{24}H_{24}NO_3$ 374.1756, found 374.1749.

(±)-2-(3-Benzoylphenyl)-*N*-(4-hydroxyphenethyl)propanamide (37). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (m, 2H), 7.66 (m, 2H), 7.60 (m, 1H), 7.47 (m, 4H), 6.82 (m, 2H), 6.68 (m, 2H), 5.48 (t, 1H, J = 6.0 Hz), 3.55 (q, 1H, J = 7.1 Hz), 3.46 (m, 1H), 3.36 (dq, 1H, J = 6.8, 13.4 Hz), 2.63 (t, 2H, J = 6.8 Hz), 1.51 (d, 3H, J = 7.2 Hz). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{24}H_{24}NO_3$ 374.1756, found 374.1750.

(*R*)-*N*-(4-Hydroxyphenethyl)-2-(6-methoxynaphthalen-2-yl)propanamide (38). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (dd, 2H, J = 5.9, 8.6 Hz), 7.57 (s, 1H), 7.28 (m, 1H), 7.16 (m, 2H), 6.74 (d, 2H, J = 8.5 Hz), 6.56 (d, 2H, J = 8.5 Hz), 5.41 (s, 1H), 3.92 (s, 3H), 3.64 (q, 1H, J = 7.2 Hz), 3.45 (dq, 1H, J = 6.6, 13.1 Hz), 3.34 (td, 1H, J = 6.7, 13.0 Hz), 2.58 (t, 2H, J = 6.8 Hz), 1.58 (d, 3H, J = 7.2 Hz). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{22}H_{24}NO_3$ 350.1756, found 350.1743.

(2*S*)-2-(3-(Hydroxy(phenyl)methyl)phenyl)-*N*-(4-hydroxyphenethyl)propanamide (39). A solution of **36** (0.016 g, 0.043 mmol) in MeOH (2 mL) was treated with $NaBH_4$ (0.002 g, 0.047 mmol). The resulting mixture was stirred for 2 h at rt. The reaction mixture was quenched with 4 mL of water and extracted with EtOAc (10 mL). The extract was washed with water (3×5 mL) and brine (1 × 5 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash-column chromatography over silica gel (Biotage SP4, 12+S column, eluting with hexane/ethyl acetate gradient 10−80%) to give **39** (0.013 g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 8H), 7.10 (dd, 1H, J = 3.8, 7.1 Hz), 6.76 (dd, 2H, J = 4.3, 8.4 Hz), 6.66 (d, 2H, J = 8.4 Hz), 5.74 (s, 1H), 5.56 (m, 1H), 3.42 (m, 2H), 3.27 (dt, 1H, J = 6.5, 13.1 Hz), 2.56 (m, 2H), 1.44 (dd, 3H, J = 2.7, 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{24}H_{26}NO_3$ 376.1913, found 376.1912.

(*S*)-2-(3-Benzylphenyl)-*N*-(4-hydroxyphenethyl)propanamide (40). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (m, 6H), 7.06 (dd, 3H, J = 7.8, 16.8 Hz), 6.79 (d, 2H, J = 8.4 Hz), 6.70 (d, 2H, J = 8.3 Hz), 5.41 (t, 1H, J = 5.5 Hz), 3.94 (s, 2H), 3.44 (m, 2H), 3.32 (td, 1H, J = 6.8, 13.0 Hz), 2.57 (m, 2H), 1.47 (d, 3H, J = 7.2 Hz). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{24}H_{26}NO_2$ 360.1964, found 360.1951.

***N*-(4-Hydroxyphenethyl)-2-(2-phenoxyphenyl)acetamide (41).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.27 (m, 4H), 7.08 (m, 2H), 6.82 (m, 5H), 6.69 (d, 2H, J = 8.5 Hz), 5.77 (s, 1H), 3.57 (s, 2H), 3.39 (q, 2H, J = 6.7 Hz), 2.61 (t, 2H, J = 6.8 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{22}H_{22}NO_3$ 348.1600, found 348.1599.

***N*-(4-Hydroxyphenethyl)-2-(3-phenoxyphenyl)acetamide (42).** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (ddd, 4H, J = 4.9, 8.2, 15.8 Hz), 7.12 (t, 1H, J = 7.4 Hz), 7.00 (m, 2H), 6.90 (m, 4H), 6.83 (s, 1H), 6.71 (d, 2H, J = 8.5 Hz), 5.41 (bs, 1H), 3.49 (s, 2H), 3.43 (dd, 2H, J = 6.6, 12.8 Hz), 2.65 (t, 2H, J = 6.8 Hz). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{22}H_{22}NO_3$ 348.1600, found 348.1592.

***N*-(4-Hydroxyphenethyl)-2-(4-phenoxyphenyl)acetamide (43).** A solution of **36** (0.040 g, 0.107 mmol) and 10% Pd/C (0.002 g) in methanol (3 mL) was stirred under H_2 for 18 h at rt and then filtered through a short pad of Celite. The solvent was removed in vacuo, and the residue was flash-chromatographed over silica gel (Biotage SP4, 12+S column, eluting with hexane/ethyl acetate gradient 10−80%) to give **43** (0.035 g, 0.097 mmol, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (t, 2H, J = 7.9 Hz), 7.11 (m, 3H), 7.01 (m, 2H), 6.94 (dd, 2H, J = 2.9, 8.5 Hz), 6.88 (dd, 2H, J = 2.6, 8.4 Hz), 6.73 (dd, 2H, J = 2.9, 8.4 Hz),

5.47 (s, 1H), 3.49 (s, 2H), 3.44 (dd, 2H, J = 6.6, 12.8 Hz), 2.66 (t, 2H, J = 6.6 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{22}H_{22}NO_3$ 348.1600, found 348.1600.

(*S*)-2-(3-Benzoylphenyl)-*N*-(2-hydroxyphenethyl)propanamide (44). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (m, 2H), 7.73 (t, 1H, J = 1.6 Hz), 7.65 (m, 1H), 7.55 (m, 4H), 7.42 (t, 1H, J = 7.6 Hz), 7.05 (m, 1H), 7.00 (dd, 1H, J = 1.6, 7.5 Hz), 6.85 (dd, 1H, J = 1.0, 8.0 Hz), 6.77 (td, 1H, J = 1.1, 7.4 Hz), 6.25 (s, 1H), 3.65 (q, 1H, J = 7.2 Hz), 3.39 (m, 2H), 2.76 (m, 2H), 1.54 (d, 3H, J = 7.3 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{24}H_{24}NO_3$ 374.1756, found 374.1746.

(*S*)-2-(3-Benzoylphenyl)-*N*-(2-methoxyphenethyl)propanamide (45). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (m, 2H), 7.67 (m, 2H), 7.60 (t, 1H, J = 7.4 Hz), 7.45 (m, 4H), 7.15 (td, 1H, J = 1.6, 7.9 Hz), 6.94 (m, 1H), 6.80 (t, 2H, J = 7.3 Hz), 5.60 (s, 1H), 3.75 (s, 3H), 3.53 (q, 1H, J = 7.1 Hz), 3.45 (m, 2H), 2.77 (m, 2H), 1.50 (d, 3H, J = 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{25}H_{26}NO_3$ 388.1913, found 388.1903.

(*S*)-2-(3-Benzoylphenyl)-*N*-(3-methoxyphenethyl)propanamide (46). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (m, 2H), 7.67 (m, 2H), 7.60 (m, 1H), 7.46 (m, 4H), 7.12 (t, 1H, J = 7.7 Hz), 6.70 (m, 1H), 6.60 (d, 2H, J = 7.3 Hz), 5.44 (s, 1H), 3.73 (s, 3H), 3.49 (m, 3H), 2.70 (t, 2H, J = 7.1 Hz), 1.52 (d, 3H, J = 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{25}H_{26}NO_3$ 388.1913, found 388.1903.

(*S*)-2-(3-Benzoylphenyl)-*N*-(4-methoxyphenethyl)propanamide (47). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, 2H, J = 7.2 Hz), 7.68 (d, 2H, J = 8.6 Hz), 7.60 (t, 1H, J = 7.4 Hz), 7.47 (m, 4H), 6.92 (d, 2H, J = 8.5 Hz), 6.75 (d, 2H, J = 8.5 Hz), 5.39 (s, 1H), 3.75 (s, 3H), 3.55 (q, 1H, J = 7.1 Hz), 3.48 (dq, 1H, J = 6.5, 13.1 Hz), 3.38 (dt, 1H, J = 6.6, 13.3 Hz), 2.66 (td, 2H, J = 2.0, 6.8 Hz), 1.52 (d, 3H, J = 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{25}H_{26}NO_3$ 388.1913, found 388.1901.

(*S*)-2-(3-Benzoylphenyl)-*N*-phenethylpropanamide (48). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, 2H, J = 1.1, 8.2 Hz), 7.67 (dd, 2H, J = 1.2, 7.2 Hz), 7.60 (t, 1H, J = 7.4 Hz), 7.47 (m, 4H), 7.19 (m, 3H), 7.02 (d, 2H, J = 6.8 Hz), 5.39 (s, 1H), 3.48 (m, 3H), 2.73 (td, 2H, J = 2.0, 6.8 Hz), 1.52 (d, 3H, J = 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{245}H_{24}NO_2$ 358.1807, found 358.1794.

(*S*)-2-(3-Benzoylphenyl)-*N*-(4-chlorophenethyl)propanamide (49). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, 2H, J = 6.9 Hz), 7.64 (m, 3H), 7.46 (dt, 4H, J = 5.9, 23.4 Hz), 7.17 (d, 2H, J = 8.1 Hz), 6.94 (d, 2H, J = 7.9 Hz), 5.44 (s, 1H), 3.45 (m, 3H), 2.70 (t, 2H, J = 6.0 Hz), 1.52 (d, 3H, J = 6.8 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{24}H_{23}NO_2Cl$ 392.1417, found 392.1414.

(*S*)-2-(3-Benzoylphenyl)-*N*-(3,4-dihydroxyphenethyl)propanamide (50). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, 2H, J = 7.8 Hz), 7.64 (dd, 3H, J = 9.6, 15.6 Hz), 7.46 (dt, 4H, J = 7.7, 27.0 Hz), 6.73 (d, 1H, J = 7.6 Hz), 6.43 (d, 2H, J = 7.6 Hz), 5.51 (s, 1H), 3.54 (m, 2H), 3.28 (dt, 1H, J = 6.6, 13.3 Hz), 2.53 (m, 2H), 1.52 (d, 3H, J = 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{24}H_{24}NO_4$ 390.1705, found 390.1703.

(*S*)-2-(3-Benzoylphenyl)-*N*-(4-hydroxy-3-methoxyphenethyl)propanamide (51). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (m, 2H), 7.69 (s, 1H), 7.66 (d, 1H, J = 7.5 Hz), 7.60 (t, 1H, J = 7.4 Hz), 7.48 (t, 3H, J = 7.6 Hz), 7.42 (t, 1H, J = 7.6 Hz), 6.75 (d, 1H, J = 8.0 Hz), 6.59 (d, 1H, J = 1.8 Hz), 6.49 (dd, 1H, J = 1.8, 8.0 Hz), 5.46 (s, 1H), 3.80 (s, 3H), 3.55 (q, 1H, J = 7.1 Hz), 3.44 (ddt, 2H, J = 6.4, 13.3, 19.4 Hz), 2.66 (t, 2H, J = 7.0 Hz), 1.52 (d, 3H, J = 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{25}H_{26}NO_4$ 404.1862, found 404.1851.

(*S*)-2-(3-Benzoylphenyl)-*N*-(3-hydroxy-4-methoxyphenethyl)propanamide (52). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (m, 2H), 7.67 (d, 2H, J = 7.6 Hz), 7.60 (t, 1H, J = 7.4 Hz), 7.47 (m, 4H), 6.67 (d, 1H, J = 8.2 Hz), 6.61 (d, 1H, J = 2.0 Hz), 6.45 (dd, 1H, J = 2.0, 8.2 Hz), 5.41 (s, 1H), 3.82 (s, 3H), 3.55 (q, 1H, J = 7.1 Hz), 3.41 (ddt, 2H, J = 6.7, 13.2, 34.1 Hz), 2.61 (td, 2H, J = 3.4, 6.7 Hz), 1.52 (d, 3H, J = 7.2 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{25}H_{26}NO_4$ 404.1862, found 404.1854.

(*S*)-2-(3-Benzoylphenyl)-*N*-((4-hydroxyphenyl)methyl)propanamide (53). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (m, 3H), 7.65 (d, 1H, J = 6.5 Hz), 7.59 (t, 2H, J = 8.4 Hz), 7.45 (m, 3H), 7.00 (d, 2H, J = 6.7 Hz), 6.71 (d, 2H, J = 6.6 Hz), 5.78 (s, 1H), 4.29 (m, 2H), 3.64 (q, 1H, J = 7.0 Hz), 1.56 (d, 3H, J = 7.1 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{23}H_{22}NO_3$ 360.1600, found 360.1593.

(*S*)-2-(3-Benzoylphenyl)-*N*-(4-hydroxyphenyl)propanamide (54). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (m, 3H), 7.62 (m, 3H), 7.46 (td, 3H, J = 3.2, 7.7 Hz), 7.32 (s, 1H), 7.19 (d, 2H, J = 8.7 Hz), 6.70 (d, 2H, J = 8.7 Hz), 3.76 (q, 1H, J = 7.0 Hz), 1.59 (d, 3H, J = 7.1 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{22}H_{20}NO_3$ 346.1443, found 346.1429.

(*S*)-(3-(1-Hydroxypropan-2-yl)phenyl)(phenyl)methanone (8). A stirred solution of **7** (0.500 g, 1.966 mmol) in THF (5 mL) at −20 °C was treated dropwise with borane−tetrahydrofuran complex (2.36 mL, 2.36 mmol), and the cooling bath was allowed to equilibrate overnight to rt. The solution was cooled to 5 °C and treated with 1 mL of methanol, diluted with 5 mL of water, and concentrated in vacuo to remove the organic solvents. The residue was diluted with water (10 mL) and dichloromethane (10 mL) and washed with $NaHCO_3$. The organic phase was dried over sodium sulfate and concentrated in vacuo. The residue was chromatographed over silica (Biotage SP4, 25+M column, eluting with hexane/ethyl acetate gradient 10−100%) to give **8** (0.470 g, 1.956 mmol, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (dd, 2H, J = 1.2, 8.2 Hz), 7.70 (s, 1H), 7.65−7.55 (m, 2H), 7.47 (dd, 3H, J = 4.7, 10.3 Hz), 7.42 (t, 1H, J = 7.6 Hz), 3.80−3.68 (m, 2H), 3.02 (sext, 1H, J = 6.9 Hz), 1.87 (s, 1H), 1.30 (d, 3H, J = 7.0 Hz).

(*S*)-(3-(1-Aminopropan-2-yl)phenyl)(phenyl)methanone (9). To a solution of **8** (0.400 g, 1.665 mmol) in dry dichloromethane (15 mL) at 0 °C under nitrogen atmosphere, triethylamine (0.7 mL, 5 mmol) was added followed by methanesulfonyl chloride (0.19 mL, 2.5 mmol) dropwise. The reaction mixture was stirred at 0 °C for 1 h and was worked up by adding water, followed by extraction with dichloromethane. The combined organic extracts were washed with water and brine and dried over anhydrous sodium sulfate. The solvent was evaporated to give the mesylate as a gum, which was taken up for the next step without any purification.

To a solution of the above mesylate (0.450 g, 1.413 mmol) in dry DMF (10 mL) under nitrogen atmosphere, sodium azide (0.092 g, 1.413 mmol) was added, and the resulting mixture was stirred at 70 °C for 2 h. The reaction mixture was allowed to cool to rt and was worked up by addition of water (20 mL) followed by extraction with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with water (5×20 mL) and brine (1 × 20 mL) and dried over sodium sulfate. The residue obtained upon evaporation of solvent was chromatographed over silica gel to get the desired azide (0.350 g, 1.319 mmol, 93% yield).

Azide from the previous step (0.350 g, 1.319 mmol) was dissolved in THF (10 mL), and triphenylphosphine (0.519 g, 1.979 mmol) was added to the solution. After being stirred for 48 h at rt, the reaction mixture was concentrated in vacuo. The residue was dissolved in 2 N sodium hydroxide (15 mL), and the product was extracted with ethyl acetate (3 × 20 mL) and washed with water (2×20 mL) followed by brine (1 × 20 mL). The organic layer was dried over sodium sulfate, evaporated to dryness, and purified by flash chromatography (Biotage SP4, 25+M silica gel column, eluting with hexane/ethyl acetate gradient 10−100%) to give **52** (0.288 g, 1.203 mmol, 91% yield). ^{1}H NMR (400 MHz, MeOD) δ 7.77 (dd, 2H, J = 3.2, 5.2 Hz), 7.69−7.59 (m, 3H), 7.56−7.45 (m, 4H), 2.94−2.79 (m, 3H), 1.30 (d, 3H, J = 6.6 Hz).

Compounds **55**−**57** were synthesized from **9** and the appropriate carboxylic acid following the general procedure mentioned above.

(*S*)-*N*-(2-(3-Benzoylphenyl)propyl)-4-hydroxybenzamide (55). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (d, 2H, J = 7.2 Hz), 7.71 (s, 1H), 7.65 (d, 1H, J = 7.3 Hz), 7.63−7.51 (m, 3H), 7.51−7.41 (m, 4H), 6.81 (d, 2H, J = 8.5 Hz), 3.87−3.72 (m, 1H), 3.53−3.40

(m, 1H), 3.23−3.10 (m, 1H), 1.37 (d, 3H, J = 7.0 Hz). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{23}H_{22}NO_3$ 360.1600, found 360.1589.

(*S*)-*N*-(2-(3-Benzoylphenyl)propyl)-3-(4-hydroxyphenyl)propanamide (56). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85−7.76 (m, 2H), 7.61 (dd, 2H, J = 2.9, 6.7 Hz), 7.55 (s, 1H), 7.49 (t, 2H, J = 7.6 Hz), 7.40 (t, 1H, J = 7.6 Hz), 7.32 (d, 1H, J = 7.7 Hz), 6.96 (d, 2H, J = 8.3 Hz), 6.69 (d, 2H, J = 8.5 Hz), 5.18 (s, 1H), 3.58 (dt, 1H, J = 6.3, 12.8 Hz), 3.21 (dd, 1H, J = 11.2, 16.2 Hz), 2.89 (d, 1H, J = 8.3 Hz), 2.81 (t, 2H, J = 7.2 Hz), 2.35 (dd, 2H, J = 5.9, 9.1 Hz), 1.22 (d, 3H, J = 7.0 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{25}H_{26}NO_3$ 388.1913, found 388.1906.

(*S*)-*N*-(2-(3-Benzoylphenyl)propyl)-4-methoxybenzamide (57). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82−7.74 (m, 2H), 7.71 (s, 1H), 7.68−7.54 (m, 4H), 7.52−7.38 (m, 4H), 6.89−6.84 (m, 2H), 6.04 (s, 1H), 3.87−3.75 (m, 4H), 3.47 (ddd, 1H, J = 5.3, 8.5, 13.6 Hz), 3.18 (dd, 1H, J = 6.8, 15.0 Hz), 1.37 (d, 3H, J = 7.0 Hz). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{24}H_{24}NO_3$ 374.1756, found 374.1746. HPLC purity 93%.

(*S*)-(3-(1-(4-Methoxybenzyloxy)propan-2-yl)phenyl)(phenyl)methanone (58). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (dd, 2H, J = 3.2, 5.2 Hz), 7.68 (s, 1H), 7.65−7.55 (m, 2H), 7.46 (t, 3H, J = 7.5 Hz), 7.40 (t, 1H, J = 7.5 Hz), 7.17 (d, 2H, J = 8.6 Hz), 6.84 (d, 2H, J = 8.6 Hz), 4.43 (s, 2H), 3.79 (s, 3H), 3.54 (m, 2H), 3.12 (dd, 1H, J = 6.9, 13.8 hz), 1.31 (d, 3H, J = 7.0 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{24}H_{25}O_3$ 361.1804, found 361.1799. HPLC purity 91%.

(*S*)-4-Nitrophenyl 2-(3-Benzoylphenyl)propylcarbamate (66). To a solution of 4-nitrophenyl chloroformate (0.111 g, 0.552 mmol) and triethylamine (0.064 mL, 0.460 mmol) in anhydrous THF (5 mL), a solution of **9** in THF (5 mL) was added dropwise at 0−5 °C under nitrogen atmosphere. The reaction mixture was then allowed to warm up to rt and was stirred for 2 h at rt. The resulting mixture was evaporated to dryness and residue chromatographed over silica gel (Biotage SP4, 25+M silica gel column, eluting with dichlomethane/ethanol gradient 0−7%) to give **66** (0.135 g, 0.334 mmol, 72.6% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26−8.17 (m, 2H), 7.85−7.78 (m, 2H), 7.72 (s, 1H), 7.66 (dt, 1H, J = 1.7, 6.8 Hz), 7.61 (t, 1H, J = 7.4 Hz), 7.48 (q, 4H, J = 7.5 Hz), 7.29−7.20 (m, 2H), 5.16 (s, 1H), 3.58 (dt, 1H, J = 6.5, 13.3 Hz), 3.42 (ddd, 1H, J = 5.6, 8.4, 13.8 Hz), 3.13 (dd, 1H, J = 6.9, 14.8 Hz), 1.37 (d, 3H, J = 7.0 Hz).

(*S*)-2-(3-Benzoylphenyl)propyl 4-Nitrophenylcarbonate (67). **67** was made following a procedure similar to that for making **66** starting from **8** in 79% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28−8.21 (m, 2H), 7.81 (d, 2H, J = 7.3 Hz), 7.75 (s, 1H), 7.68 (d, 1H, J = 7.4 Hz), 7.63−7.57 (m, 1H), 7.52−7.47 (m, 4H), 7.35−7.28 (m, 2H), 4.41 (qd, 2H, J = 7.0, 10.6 Hz), 3.32 (dd, 1H, J = 7.0, 14.0 Hz), 1.42 (d, 3H, J = 7.0 Hz).

(*S*)-1-(2-(3-Benzoylphenyl)propyl)-3-(4-hydroxyphenyl)urea (59). To a solution of **66** (0.020 g, 0.049 mmol) and 4-aminophenol (6.48 mg, 0.059 mmol) in anhydrous THF (2 mL), triethylamine (8.27 μL, 0.059 mmol) was added. The resulting mixture was stirred at rt for 15 h and then evaporated to dryness and residue chromatographed over silica gel (Biotage SP4, 25+M silica gel column, eluting with dichlomethane/ethanol gradient 0−7%) to **59** (0.011 g, 0.029 mmol, 59.4% yield). ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ 7.79 (d, 2H, J = 8.2 Hz), 7.61 (dd, 3H, J = 7.1, 14.5 Hz), 7.55 − 7.40 (m, 4H), 7.00 (d, 2H, J = 8.8 Hz), 6.77 (d, 2H, J = 8.8 Hz), 3.48 (dd, 1H, J = 6.4, 13.4 Hz), 3.26 (dd, 1H, J = 8.5, 13.5 Hz), 3.04 (dd, 1H, J = 7.0, 14.4 Hz), 1.31 (d, 3H, J = 7.0 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{23}H_{23}N_2O_3$ 375.1709, found 375.1706. HPLC purity 87%.

(*S*)-1-(2-(3-Benzoylphenyl)propyl)-3-(4-hydroxyphenethyl)urea (60). 60 was synthesized by following the procedure for synthesis of **59** using **6**. ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ 7.82−7.75 (m, 2H), 7.64−7.54 (m, 3H), 7.47 (t, 2H, J = 7.7 Hz), 7.43−7.35 (m, 2H), 6.91 (d, 2H, J = 8.4 Hz), 6.71 (d, 2H, J = 8.4 Hz), 3.42 (dd, 1H, J = 6.2, 13.5 Hz), 3.29 (t, 2H, J = 6.7 Hz), 3.18 (dd, 1H, J = 8.3, 13.4 Hz), 2.95 (dd, 1H, J = 6.9, 14.5 Hz), 2.62 (t, 2H, J = 6.7 Hz), 1.25 (d, 3H, J = 7.0 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{25}H_{27}N_2O_3$ 403.2022, found 403.2015. HPLC purity 91%.

Compounds **61** and **62** were synthesized from **67** following the procedure for synthesis of **59** using the appropriate amine.

(*S*)-2-(3-Benzoylphenyl)propyl 4-hydroxyphenylcarbamate (61). ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD) δ 7.80 (d, 2H, J = 7.2 Hz), 7.72 (s, 1H), 7.65 (d, 1H, J = 7.4 Hz), 7.59 (t, 1H, J = 7.4 Hz), 7.54−7.38 (m, 4H), 7.19 (m, 2H), 6.75 (d, 2H, J = 8.3 Hz), 6.43 (bs, 1H), 4.33−4.21 (m, 2H), 3.22 (dd, 1H, J = 7.0, 14.0 Hz), 1.35 (d, 3H, J = 7.1 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{23}H_{22}NO_4$ 376.1549, found 376.1554.

(*S*)-2-(3-Benzoylphenyl)propyl 4-Hydroxyphenethylcarbamate (62). ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ 7.80 (d, 2H, J = 7.4 Hz), 7.71−7.55 (m, 3H), 7.52−7.37 (m, 4H), 6.98 (d, 2H, J = 8.0 Hz), 6.74 (d, 2H, J = 8.3 Hz), 4.65 (s, 1H), 4.17 (d, 2H, J = 7.0 Hz), 3.35 (dd, 2H, J = 6.4, 12.7 Hz), 3.20 − 3.08 (m, 1H), 2.69 (t, 2H, J = 6.7 Hz), 1.30 (d, 3H, J = 6.9 Hz). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{25}H_{26}NO_4$ 404.1862, found 404.1854.

4-(2-(1-Propylamino)ethyl)phenol (11). To a solution of 4-hydroxyphenethyl bromide (0.200 g, 0.995 mmol) in acetonitrile (5 mL), propylamine (0.817 mL, 9.95 mmol) was added, followed by DIPEA (0.520 mL, 2.98 mmol). The resulting solution was stirred at 60 °C for 6 h. The reaction mixture was allowed to cool to rt and was filtered. The solvent was removed in vacuo, and residue was flash chomatographed over silica gel (Biotage SP4, 25+S silica gel column, eluting with hexan and ethyl acetate gradient 10−100%) to give **11** (0.150 g, 0.837 mmol, 84% yield). ^{1}H NMR (400 MHz, MeOD) δ 7.13 (d, 2H, J = 8.6 Hz), 6.83−6.74 (m, 2H), 3.23−3.16 (m, 2H), 3.04−2.96 (m, 2H), 2.93 (dd, 2H, J = 6.9, 9.3 Hz), 1.74 (dq, 2H, J = 7.5, 15.1 Hz), 1.04 (t, 3H, J = 7.5 Hz).

4-(2-(2-Methoxyethylamino)ethyl)phenol (12). 12 was synthesized in a manner similar to **11** starting from 4-hydroxyphenethyl bromide and 2-methoxyethylamine. ^{1}H NMR (400 MHz, $CDCl_3$+CD_3OD) δ 7.00 (d, 2H, J = 8.4 Hz), 6.69 (d, 2H, J = 8.5 Hz), 5.43 (s, 1H), 3.50 (dd, 2H, J = 5.8, 10.9 Hz), 3.30 (s, 3H), 2.89 (t, 2H, J = 7.0 Hz), 2.83 (t, 2H, J = 5.1 Hz), 2.75 (t, 2H, J = 7.1 Hz).

4,4′-(2,2′-Azanediylbis(ethane-2,1-diyl))diphenol (13). 13 was synthesized in a manner similar to **11** starting from 4-hydroxyphenethyl bromide and tyramine. ^{1}H NMR (400 MHz, $CDCl_3$ +CD_3OD) δ 6.94 (dd, 2H, J = 4.6, 6.6 Hz), 6.77−6.68 (m, 2H), 2.83 (t, 2H, J = 6.9 Hz), 2.70 (t, 2H, J = 7.0 Hz).

(*S*)-2-(3-Benzoylphenyl)-*N*-(4-hydroxyphenethyl)-*N*-propylpropanamide (63). 63 was synthesized from **7** and **11** following the general procedure mentioned above. ^{1}H NMR (400 MHz, $CDCl_3$) (a mixture of rotamers) δ 7.84−7.73 (m, 2H), 7.72−7.51 (m, 4H), 7.51−7.37 (m, 3H), 7.01−6.89 (m, 2H), 6.78 (d, 1H, J = 8.2 Hz), 6.70 (d, 1H, J = 8.1 Hz), 6.00 and 5.61 (2 s, 1H, −OH), 3.97−3.69 (m, 1H), 3.45−2.85 (m, 4H), 2.81−2.52 (m, 2H), 1.58−1.24 (m, 5H with two overlapping doublets of rotamers), 0.91−0.77 (m, 3H). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{27}H_{30}NO_3$ 416.2226, found 416.2222.

(*S*)-2-(3-Benzoylphenyl)-*N*-(4-hydroxyphenethyl)-*N*-(2-methoxyethyl)propanamide (64). 64 was synthesized from **7** and **12** following the general procedure mentioned above. ^{1}H NMR (400 MHz, $CDCl_3$) (a mixture of rotamers) δ 7.83−7.74 (m, 2H), 7.72−7.51 (m, 4H), 7.44 (m, 3H), 6.94 (dd, 2H, J = 2.1, 8.5 Hz), 6.77 (d, 1H, J = 8.5 Hz), 6.67 (d, 1H, J = 8.5 Hz), 5.93 and 5.63 (2s, 1H), 4.17−3.01 (m, 9H, with overlapping singlet at 3.28), 2.80−2.53 (m, 2H), 1.47 and 1.30 (2d, J = 6.9, 3H). HRMS (ESI $(M + H)^+$ m/z) calcd for $C_{27}H_{30}NO_4$ 432.2175, found 432.2173.

(*S*)-2-(3-Benzoylphenyl)-*N*,*N*-bis(4-hydroxyphenethyl)propanamide (65). 65 was synthesized from **7** and **13** following the general procedure mentioned above. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, 2H, J = 7.1 Hz), 7.65−7.55 (m, 3H), 7.49−7.39 (m, 4H), 6.93 (dd, J = 8.4, 14.7, 4H), 6.76 (d, 2H, J = 8.5 Hz), 6.67 (d, 2H, J = 8.5 Hz), 3.85−3.74 (m, 1H), 3.57 (q, 1H, J = 6.7 Hz), 3.45−3.35 (m, 1H), 3.28−3.19 (m, 1H), 3.17−3.07 (m, 1H), 2.82−2.63 (m, 3H),

2.58−2.49 (m, 1H), 1.29 (d, $J = 6.9$, 4H). HRMS (ESI $(M+H)^+$ m/z) calcd for $C_{32}H_{32}NO_4$ 494.2331, found 494.2311.

Gli-Mediated Transcription Reporter Assay in C3H10T1/2 Cells with Exogenous h*Gli1* or ΔNh*Gli2*. C3H10/T1/2 cells were plated at 2.5×10^5 cells/dish in two 60 mm culture dishes in 5 mL of basal medium Eagle (BME) (Invitrogen Corporation, Carlsbad, CA) containing 2 mM L-glutamine and 10% fetal bovine serum (FBS, Hyclone Laboratories, Logan, UT). The cells were maintained in 5% CO_2 at 37 °C in a humidified incubator. After 24 h, the cells were transfected at approximately 75% confluence by replacing the media with 5 mL of BME (10% FBS) containing the DNA mixture of GliLuc-BS (250 ng/mL), hGli1 (250 ng/mL), and pRL-TK (12.5 ng/mL) (Promega, Madison, WI) and the transfection reagent Fugene6 (1:3 w/v of DNA) (F. Hoffmann-La Roche, Basel, Switzerland) and incubated overnight. A simultaneous control was transfected with empty vector, pcDNA3, GliLuc, pRL-TK, and Fugene6 in similar ratios as used for hGli1. Eighteen hours after transfection, the cells were trypsinized and reconstituted in 10 mL of BME (10% FBS) and plated in a white 96-well cell culture plate at 100 μL/well. Six hours after plating, the media in the wells were replaced with 100 μL of BME (10% FBS) containing the compound or the DMSO control. After exposure of the cells to compound for 24 h at 37 °C in 5% CO_2, the media were aspirated, and the cells were lysed with 20 μL/well of passive lysis buffer (1×) by placing the plate on a shaking platform for 15 min at 600 rpm. The luciferase activity was determined using dual-luciferase reporter assay system (Promega) according to manufacturer's instructions. The activity was determined by dividing the luminescence of the luciferase by that of Renilla.

To enhance the luciferase signal in the assay readout of Gli2 transcription, we used the ΔNhGli2, an hGli2 construct from which the amino-terminal region encoding a transcription repressor domain was deleted.[29] The assay for ΔNhGli2 was carried out following the protocol for the hGli1-transactivation assay; however, hGli1 was replaced with ΔNhGli2.

Gli-Mediated Transcription Reporter Assay in Rh30 Cells. Rh30 cells were plated at 3×10^5 cells/dish in two 60 mm culture dishes in 5 mL of RPMI-1640 (ATCC, Manassas, VA) containing 10% FBS (Hyclone Laboratories) and were maintained in 5% CO_2 at 37 °C in a humidified incubator. After 24 h, the cells were transfected at approximately 70% confluence by replacing the media with 5 mL of RPMI-1640 (10% FBS) containing the DNA mixture of GliLuc-BS (500 ng/mL) and pRL-CMV (2.5 ng/mL) (Promega) and Fugene6 (1:3 w/v of DNA) (F. Hoffmann-La Roche, Basel, Switzerland) and incubated overnight. Six hours after transfection, the cells were trypsinized and reconstituted in 10 mL of RPMI-1640 (10% FBS) and plated in a white 96-well cell culture plate at 100 μL/well. Eighteen hours after plating, the media were replaced with 100 μL of RPMI-1640 (10% FBS) containing the compound or the DMSO control. After cells were exposed to compound for 24 h at 37 °C in 5% CO_2, the media were aspirated, and the cells were lysed with 20 μL/well of passive lysis buffer (1×) by placing the plate on a shaking platform for 15 min at 600 rpm. The luciferase activity was determined using dual-luciferase reporter assay system (Promega) according to the manufacturer's instuctions. The activity was determined by dividing the luminescence of luciferase by that of Renilla.

Cell Viability Assay. The cells were plated in the appropriate medium in a 384-well plate 24 h before the addition of the compounds. The 10 mM stock solution of each compound in DMSO was diluted in EMEM media to prepare 160 μM solution (4×), and 10 μL of that solution was added in triplicate to get a final concentration of 40 μM/well. The cells were allowed to incubate at 37 °C for 68 h. Alamar Blue (4 μL) (Biotium, Inc., Hayward, CA) was added to each well, and the plates were incubated for 4 h at 37 °C. The fluorescence was measured with an excitation wavelength at 510 nm and emission wavelength at 590 nm using an EnVision multilabel plate reader (PerkinElmer, Waltham, MA). The cell viability was determined by comparing the fluorescence of experimental wells with that of the DMSO control wells.

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Supporting Information Available: Scatter plots of activity vs the calculated log *P* or tPSA values, the cell viability data for each compound on each cell line, HPLC traces for the compounds, data of stability test, counterassays for nonspecific transcription inhibition, and detail of statistic analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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