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

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Abstract

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 IC₅₀ 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 upregulated expression of the *Gli1* gene. These compounds decreased the viability of several cancer cell lines but were less active in that of noncancerous BJ-*hTERT* cells.

Keywords

anticancer; Sonic Hedgehog; Gli; benzophenone

Introduction

The Sonic hedgehog (Shh) -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

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Supporting Information Available: The scatter plots of activity vs the calculated logP or tPSA values, the cell viability data for each compound on each cell line, HPLC traces for the compounds, data of stability test, counter-assays for non-specific transcription inhibition, and detail of statistic analysis, are available online, free of charge at <http://pubs.acs.org>.

is not the only activator of Gli1-mediated transcription; other regulatory mechanisms downstream of Smo also upregulate 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 tumorigenesis.^{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 drugs²⁴ tolerated well by pediatric patients who need hedgehog activity for somatic development and growth.

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 Gli1-mediated transcription based on **5** by replacing the compound's pyrazoline moiety while retaining a tyramine amide moiety that is an essential pharmacophore for **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**.

Results and Discussion

We started our SAR investigation by replacing the head-part of **5** (Figure 1). To assay compounds for selective inhibition of Gli1-mediated transcription, we used C3H10T1/2 mouse embryo fibroblasts with exogenously transfected vectors encoding human Gli1 and a Gli-luciferase reporter vector²⁷. Because the Gli-reporter activities in these cells are activated solely by the exogenous Gli1, compounds that downregulate 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) 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 that of **34** at 20 μ M and equivalent activity at 40 μ M. To evaluate the effect of the chirality on the methylene spacer, we compared the activity of **35** with its (*R*)-enantiomer **38** and that of **36** with 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 of 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).

Next, we focused on **36** to investigate the SAR of the tail-part, because this compound has high activity and minimal toxicity as compared to **32** towards 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 μ M when the hydroxyl group was moved to *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 analog **50** afforded a higher activity than the phenol analog **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 group 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. (Figure 4 and Figure 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 amide carbonyl 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 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 non-cancerous 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 values of logP or total polar surface area for each compound (Supplemental 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 confirmed to overexpress the *exogenous* Gli1 protein and it is not reduced by **36** or **63** (Supplemental 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** (Supplemental Figure 6), confirming that they are not inhibitors of luciferase reporter.

Selected compounds were evaluated for their selectivity in inhibiting the Gli1-mediated transcription over Gli2-mediated transcription by determining their IC₅₀ values (Figure 8 and Table 3). The compounds showed 3 to 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 hours. (Supplemental 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 cotransfection 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 cells in which growth control is *Gli1*-dependent, such as medulloblastoma progenitor cells.¹⁷

To determine whether inhibitors of Gli1-mediated transcription inhibit the growth of cancer cells, we tested them 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 fibroblasts³⁰, Figure 10H). This finding suggests that inhibitors of Gli1-mediated transcription could suppress the growth of cancer cells. However, it is difficult to observe simple relationship between reduction of cell viability and inhibition of Gli1-mediated transcription, likely due to a non-specific 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 *exogenous* Gli1 protein expression in the C3H10T1/2 cells (Supplementary Figure 5) but not *endogenous* 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 Rh30³, MDA-MB231³¹, Daoy^{32, 33}, and DU145³⁴ cell lines. However, it is well-known that Gli1 protein is down-regulated post-translationally^{32, 35} even when *Gli1* mRNA is expressed³⁶, 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 non-overexpression) 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, downregulated endogenous Gli-mediated transcription in Rh30 cells, and demonstrated inhibition selectivity of Gli1-mediated transcription that approximately 3 times greater than that of Gli2-mediated transcription. Cell viability is reduced by analogues of those compounds in cancer cells. These compounds might be the basis to design potential lead compounds that function as anticancer agents by selectively inhibiting Gli1-mediated transcription.

Experimental Section

General Procedure

Compounds **14–38**, **41–54**, and **55–56** were synthesized as follows: A mixture appropriate carboxylic acid (1 eq), HBTU (2.5 eq), and DIPEA (3 eq) in DMF (0.3 mL) was allowed to stand for 30 min at rt. Appropriate amine (3.0 eq) 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 sulphate. 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 μ m (4.6 $\times$ 50 mm) column by running a 0 to 95% gradient for Water (+ 0.05% TFA)/MeOH (Supplemental Figure 3). All the compounds showed $\geq$ 95% purity, unless otherwise mentioned.

N-(4-Hydroxyphenethyl)benzamide (14)— ^1H NMR (400 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$) 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 $\text{C}_{15}\text{H}_{16}\text{NO}_2$ 242.1181, found 242.1175.

4-Hydroxy-N-(4-hydroxyphenethyl)benzamide (15)— ^1H NMR (400 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$) 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 $\text{C}_{15}\text{H}_{16}\text{NO}_3$ 258.1130, found 258.1125. HPLC purity 93%.

4-Chloro-N-(4-hydroxyphenethyl)benzamide (16)— ^1H NMR (400 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$) 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 $\text{C}_{15}\text{H}_{15}\text{NO}_2\text{Cl}$ 276.0791, found 276.0781.

N-(4-Hydroxyphenethyl)biphenyl-4-carboxamide (17)— ^1H NMR (400 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$) 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 $\text{C}_{21}\text{H}_{20}\text{NO}_2$ 318.1494, found 318.1491.

4-Benzoyl-N-(4-hydroxyphenethyl)benzamide (18)— ^1H NMR (400 MHz, $\text{CDCl}_3 + \text{CD}_3\text{OD}$) 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 $\text{C}_{22}\text{H}_{20}\text{NO}_3$ 346.1443, found 346.1436.

N-(4-Hydroxyphenethyl)-2-phenylacetamide (19)— ^1H 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 $\text{C}_{16}\text{H}_{18}\text{NO}_2$ 256.1338, found 256.1335.

N-(4-Hydroxyphenethyl)-2-*p*-tolylacetamide (20)— ^1H 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 C₁₇H₂₀NO₂ 270.1494, found 270.1483.

***N*-(4-Hydroxyphenethyl)-2-(4-methoxyphenyl)acetamide (21)**—¹H NMR (400 MHz, CDCl₃) 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 C₁₇H₂₀NO₃ 286.1443, found 286.1436.

2-(2,5-Dimethoxyphenyl)-*N*-(4-hydroxyphenethyl)acetamide (22)—¹H NMR (400 MHz, CDCl₃) 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 C₁₈H₂₂NO₄ 316.1549, found 316.1545.

2-(3-Bromophenyl)-*N*-(4-hydroxyphenethyl)acetamide (23)—¹H NMR (400 MHz, CDCl₃) 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 C₁₆H₁₆BrNO₂Na 356.0262, found 356.0255.

***N*-(4-Hydroxyphenethyl)-2-(naphthalen-2-yl)acetamide (24)**—¹H NMR (400 MHz, CDCl₃) 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 C₂₀H₂₀NO₂ 306.1494, found 306.1486.

***N*-(4-Hydroxyphenethyl)-2,2-diphenylacetamide (25)**—¹H NMR (400 MHz, CDCl₃) 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 C₂₂H₂₂NO₂ 332.1651, found 332.1642.

***N*-(4-Hydroxyphenethyl)-2-(6-methoxy-3-oxo-2,3-dihydro-1*H*-inden-1-yl)acetamide (26)**—¹H NMR (400 MHz, CDCl₃) 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 C₂₀H₂₂NO₄ 340.1549, found 340.1533.

***N*-(4-Hydroxyphenethyl)-3-phenylpropanamide (27)**—¹H NMR (400 MHz, CDCl₃) 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 C₁₇H₂₀NO₂ 270.1494, found 270.1504.

***N*-(4-Hydroxyphenethyl)cinnamamide (28)**—¹H NMR (400 MHz, CDCl₃) 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 C₁₇H₁₈NO₂ 268.1338, found 268.1327. HPLC purity 92%.

(1*SR*, 2*SR*)-*N*-(4-Hydroxyphenethyl)-2-phenylcyclopropanecarboxamide (29)—¹H NMR (400 MHz, CDCl₃) 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 C₁₈H₂₀NO₂ 282.1494, found 282.1491.

***N*-(4-Hydroxyphenethyl)-1,2,3,4-tetrahydronaphthalene-2-carboxamide (30)**

—¹H NMR (400 MHz, CDCl₃) 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 C₁₉H₂₂NO₂ 296.1651, found 296.1641.

***N*-(4-Hydroxyphenethyl)-2-(5-methoxy-1*H*-indol-3-yl)acetamide (31)**—¹H NMR (400 MHz, CDCl₃) 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 C₁₉H₂₁N₂O₃ 325.1552, found 325.1545.

2-(5-(Benzyloxy)-1*H*-indol-3-yl)-*N*-(4-hydroxyphenethyl)acetamide (32)—¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₂₅N₂O₃ 401.1865, found 401.1855.

2-(1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl)-*N*-(4-hydroxyphenethyl) acetamide (33)—¹H NMR (400 MHz, CDCl₃ + CD₃OD) 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 C₂₇H₂₆N₂O₄Cl 477.1581, found 477.1571.

***N*-(4-Hydroxyphenethyl)-2-(4-isobutylphenyl)propanamide (34)**—¹H NMR (400 MHz, CDCl₃) 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 C₂₁H₂₈NO₂ 326.2120, found 326.2109.

(*S*)-*N*-(4-Hydroxyphenethyl)-2-(6-methoxynaphthalen-2-yl)propanamide (35)—¹H NMR (400 MHz, CDCl₃) 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 C₂₂H₂₄NO₃ 350.1756, found 350.1754.

(*S*)-2-(3-Benzoylphenyl)-*N*-(4-hydroxyphenethyl)propanamide (36)—¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₄NO₃ 374.1756, found 374.1749.

(±)-2-(3-Benzoylphenyl)-*N*-(4-hydroxyphenethyl)propanamide (37)—¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₄NO₃ 374.1756, found 374.1750.

(R)-N-(4-Hydroxyphenethyl)-2-(6-methoxynaphthalen-2-yl)propanamide (38)

—¹H NMR (400 MHz, CDCl₃) 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 C₂₂H₂₄NO₃ 350.1756, found 350.1743.

(2S)-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 NaBH₄ (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 Na₂SO₄, 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%). ¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₆NO₃ 376.1913, found 376.1912.

(S)-2-(3-Benzylphenyl)-N-(4-hydroxyphenethyl)propanamide (40)

—¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₆NO₂ 360.1964, found 360.1951.

N-(4-Hydroxyphenethyl)-2-(2-phenoxyphenyl)acetamide (41)

—¹H NMR (400 MHz, CDCl₃) 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 C₂₂H₂₂NO₃ 348.1600, found 348.1599.

N-(4-Hydroxyphenethyl)-2-(3-phenoxyphenyl)acetamide (42)

—¹H NMR (400 MHz, CDCl₃) 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 C₂₂H₂₂NO₃ 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 H₂ 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). ¹H NMR (400 MHz, CDCl₃) 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 C₂₂H₂₂NO₃ 348.1600, found 348.1600.

(S)-2-(3-Benzoylphenyl)-N-(2-hydroxyphenethyl)propanamide (44)

—¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₄NO₃ 374.1756, found 374.1746.

(S)-2-(3-Benzoylphenyl)-N-(2-methoxyphenethyl)propanamide (45)—¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₂₆NO₃ 388.1913, found 388.1903.

(S)-2-(3-Benzoylphenyl)-N-(3-methoxyphenethyl)propanamide (46)—¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₂₆NO₃ 388.1913, found 388.1903.

(S)-2-(3-Benzoylphenyl)-N-(4-methoxyphenethyl)propanamide (47)—¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₂₆NO₃ 388.1913, found 388.1901.

(S)-2-(3-Benzoylphenyl)-N-phenethylpropanamide (48)—¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₄NO₂ 358.1807, found 358.1794.

(S)-2-(3-Benzoylphenyl)-N-(4-chlorophenethyl)propanamide (49)—¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₃NO₂Cl 392.1417, found 392.1414.

(S)-2-(3-Benzoylphenyl)-N-(3,4-dihydroxyphenethyl)propanamide (50)—¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₄NO₄ 390.1705, found 390.1703.

(S)-2-(3-Benzoylphenyl)-N-(4-hydroxy-3-methoxyphenethyl)propanamide (51)—¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₂₆NO₄ 404.1862, found 404.1851.

(S)-2-(3-Benzoylphenyl)-N-(3-hydroxy-4-methoxyphenethyl)propanamide (52)—¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₂₆NO₄ 404.1862, found 404.1854.

(S)-2-(3-Benzoylphenyl)-N-((4-hydroxyphenyl)methyl)propanamide (53)—¹H NMR (400 MHz, CDCl₃) 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 C₂₃H₂₂NO₃ 360.1600, found 360.1593.

(S)-2-(3-Benzoylphenyl)-N-(4-hydroxyphenyl)propanamide (54)—¹H NMR (400 MHz, CDCl₃) 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 C₂₂H₂₀NO₃ 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 NaHCO₃. 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). ¹H NMR (400 MHz, CDCl₃) 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. 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 sulphate. 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 2N 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). ¹H 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)—¹H NMR (400 MHz, CDCl₃) 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 C₂₃H₂₂NO₃ 360.1600, found 360.1589.

(S)-N-(2-(3-Benzoylphenyl)propyl)-3-(4-hydroxyphenyl)propanamide (56)—¹H NMR (400 MHz, CDCl₃) 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 C₂₅H₂₆NO₃ 388.1913, found 388.1906.

(S)-N-(2-(3-Benzoylphenyl)propyl)-4-methoxybenzamide (57)—¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₄NO₃ 374.1756, found 374.1746. HPLC purity 93%.

(S)-(3-(1-(4-Methoxybenzyloxy)propan-2-yl)phenyl)(phenyl)methanone (58)—¹H NMR (400 MHz, CDCl₃) 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 C₂₄H₂₅O₃ 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 °C to 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 silicagel (Biotage SP4, 25+M silica gel column, eluting with dichloromethane ethanol gradient 0%–7%) to give **66** (0.135 g, 0.334 mmol, 72.6% yield). ¹H NMR (400 MHz, CDCl₃) 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-nitrophenyl carbonate (67)—was made following a procedure similar to that for making **66** starting from **8** in 79% yield. ¹H NMR (400 MHz, CDCl₃) 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 dichloromethane ethanol gradient 0%–7%) to **59** (0.011 g, 0.029 mmol, 59.4% yield). ¹H NMR (400 MHz, CDCl₃ + CD₃OD) 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 C₂₃H₂₃N₂O₃ 375.1709, found 375.1706. HPLC purity 87%.

(S)-1-(2-(3-Benzoylphenyl)propyl)-3-(4-hydroxyphenethyl)urea (60)—was synthesized by following the procedure for synthesis of **59** using **6**. ¹H NMR (400 MHz, CDCl₃ + CD₃OD) 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 C₂₅H₂₇N₂O₃ 403.2022, found 403.2015. HPLC purity 91%.

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

(S)-2-(3-Benzoylphenyl)propyl 4-hydroxyphenylcarbamate (61)—¹H NMR (400 MHz, CDCl₃ + CD₃OD) 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 C₂₃H₂₂NO₄ 376.1549, found 376.1554.

(S)-2-(3-Benzoylphenyl)propyl 4-hydroxyphenethylcarbamate (62)—¹H NMR (400 MHz, CDCl₃ + CD₃OD) 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 C₂₅H₂₆NO₄ 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 chromatographed over silica gel (Biotage SP4, 25+S silica gel column, eluting with hexan and ethylacetate gradient 10%-100%) to give **11** (0.150 g, 0.837 mmol, 84% yield). ¹H 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)—was synthesized in a manner similar to **11** starting from 4-hydroxyphenethyl bromide and 2-methoxyethylamine. ¹H NMR (400 MHz, CDCl₃ + CD₃OD) 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)—was synthesized in a manner similar to **11** starting from 4-hydroxyphenethyl bromide and tyramine. ¹H NMR (400 MHz, CDCl₃ + CD₃OD) 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)—was synthesized from **7** and **11** following the general procedure mentioned above. ¹H NMR (400 MHz, CDCl₃) (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 C₂₇H₃₀NO₃ 416.2226, found 416.2222.

(S)-2-(3-Benzoylphenyl)-N-(4-hydroxyphenethyl)-N-(2-methoxyethyl)propanamide (64)—was synthesized from **7** and **12** following the general procedure mentioned above. ¹H NMR (400 MHz, CDCl₃) (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 C₂₇H₃₀NO₄ 432.2175, found 432.2173.

(S)-2-(3-Benzoylphenyl)-N,N-bis(4-hydroxyphenethyl)propanamide (65)—was synthesized from **7** and **13** following the general procedure mentioned above. ¹H NMR (400 MHz, CDCl₃) 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 C₃₂H₃₂NO₄ 494.2331, found 494.2311.

Gli-mediated transcription reporter assay in C3H10T1/2 cells with exogenous *Gli1* or *Gli2*

C3H10T1/2 cells were plated at 2.5×10⁵ 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% CO₂ 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 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 Eugene6 (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 Eugene6 in similar ratios as used for hGli1. Eighteen hours after transfection, the cells were trypsinized and reconstituted in 10 mL 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 was replaced with 100 μL BME (10% FBS) containing the compound or the DMSO control. After exposing the cells to compound for 24 h at 37 °C in 5% CO₂, the media was 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 deleted²⁹. 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 3×10⁵ cells/dish in two 60-mm culture dishes in 5 mL RPMI-1640 (ATCC, Manassas, VA) containing 10% FBS (Hyclone Laboratories) and were maintained in 5% CO₂ 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 RPMI-1640 (10% FBS) containing the DNA mixture of GliLuc-BS (500 ng/mL) and pRL-CMV (2.5 ng/mL) (Promega), and Eugene6 (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 RPMI-1640 (10% FBS) and plated in a white 96-well cell culture plate at 100 μ L/well. Eighteen hours after plating, the media was replaced with 100 μ L 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% CO₂, the media was aspirated, and the cells were lysed with 20 μ L/well of Passive Lysis Buffer (1 $\times$) 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 instructions. 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- μ M solution (4 $\times$), and 10 μ L of that solution was added in triplicate to get a final concentration of 40 μ M/well. The cells were allowed to incubate at 37 °C for 68 h. Alamar Blue (4 μ L) (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 excitation wavelength at 510 nm and emission wavelength at 590 nm using an EnVision Multilabel Plate Reader (PerkinElmer, Waltham, MA, USA). The cell viability was determined by comparing the fluorescence of experimental wells with that of the DMSO control wells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

Gli1	glioma-associated oncogene homolog 1
Shh	Sonic Hedgehog
Ptch	Patched
Smo	Smoothed
HBTU	2-(1 <i>H</i> -benzo[<i>d</i>][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate
DIPEA	<i>N,N</i> -Diisopropylethylamine
rt	room temperature

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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**.

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

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 plot) or 40 μM (blue plot) of the test compound (**5**, **31–43**). DMSO control = 0%. Error bars represent the SEs of triplicated data.

36 R₁ = 4-OH, R₂ = H

44 R₁ = 2-OH, R₂ = H

48 R₁, R₂ = H

49 R₁ = 4-Cl, R₂ = H

45 R₁ = 2-OMe, R₂ = H

46 R₁ = 3-OMe, R₂ = H

47 R₁ = 4-OMe, R₂ = H

50 R₁ = 3-OH, R₂ = 4-OH

51 R₁ = 3-OMe, R₂ = 4-OH

52 R₁ = 3-OH, R₂ = 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 plot) or 40 μM (blue plot) of the test compound (36, 44–52). DMSO control = 0%. Error bars represent the SEs of triplicated data.

59

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 plot) or 40 μM (blue plot) of the test compound (36, 53–57, 59–65). DMSO control = 0%. Error bars represent the SEs of triplicated data.

Figure 7.
Summary of the structure-activity relationships.

Figure 8. Dose-response curve of Gli-reporter activity in Gli1-transfected (A) or Δ NGli2²⁹-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 triplicated data. Response curve fittings were calculated by Prism software (GraphPad). IC₅₀ in these curves is summarized in Table 3.

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 triplicated data.

Figure 10.

Scatter plots of the inhibition% of Gli1-mediated transcription in the C3H10/T1/2 cells 24 h after treatment with 40 μ M of each compound vs. the cell viability% of each cell line 72 h after treatment in an AlmarBlue assay. Each plot represents each single compound shown in Figure 3, Figure 5, and Figure 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 Supplement Figure 2.

Scheme 1a.

^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) $\text{BH}_3\text{-THF}$, THF, -20°C – 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) $\text{R}_1\text{-Br}$, NaH , DMF, rt; (i) 4-nitrophenyl chloroformate, Et_3N , THF, 0°C – rt; (j) R_1NH_2 , Et_3N , THF, 0°C – 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 Table 1 and Table 2 and in Figure 2 and Figure 4.

Table 1Compounds with different R groups at the head-part of **5**

	R	
5	racemic	37
31		38

R

32

39

R

33

40

R

34

41

R

35

42

36

43

Table 2Analogues for studying SARs at the linker-part of **36**.

R

53

60

54

61

R

55

62

56

63

57

64

58

65

59

Table 3

The IC₅₀ (μM) and 95% confidence intervals (in parenthesis) of selected compounds for inhibiting transcriptional activation by exogenous human Gli1 or ΔNGli2²⁹ in C3H10T1/2 cells 24 h after addition of the test compound (Figure 8).

	Gli1	Gli2
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)