Published in final edited form as:

J Med Chem. 2012 August 9; 55(15): 6738–6750. doi:10.1021/jm300752n.

Design and *in vitro* activities of *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]heteroarylsulfonamides, novel small molecule Hypoxia Inducible Factor-1 (HIF-1) pathway inhibitors and anti-cancer agents

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Abstract

The Hypoxia Inducible Factor (HIF) pathway is an attractive target for cancer as it controls tumor adaptation to growth under hypoxia and mediates chemo- and radiation resistance. We previously discovered 3,4-dimethoxy-*N*-[(2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-phenylbenzenesulfonamide, as a novel small molecule HIF-1 pathway inhibitor in a high-throughput cell-based assay, but its *in vivo* delivery is hampered by poor aqueous solubility (0.009 μ M in water; $\log P_{7.4}$ 3.7). Here we describe the synthesis of twelve *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]heteroarylsulfonamides, which were designed to possess optimal lipophilicities and aqueous solubilities by *in silico* calculations. Experimental $\log P_{7.4}$ values of 8 of the 12 new analogs ranged from 1.2 ~ 3.1. Aqueous solubilities of 3 analogs were measured, among which the most soluble *N*-[(8-methoxy-2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(propan-2-yl)pyridine-2-sulfonamide had an aqueous solubility of 80 μ M, e.g. a solubility improvement of ~9,000-fold. The pharmacological optimization had minimal impact on drug efficacy as the compounds retained IC₅₀ values at or below 5 μ M in our HIF-dependent reporter assay.

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Supporting Information **Available:** NMR, HPLC, TLC, metabolic stability curve, and cytotoxicity curves of twelve *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]heteroarylsulfonamides are available free of charge via the Internet at <http://pubs.acs.org>.

Keywords

drug discovery; HIF pathway inhibitors; anticancer drug; Hypoxia Inducible Factor; *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]heteroarylsulfonamides; a HRE-mediated luciferase assay; HIF-1 α western blotting assay; *logP*_{7.4}; drug aqueous solubility; sulforhodamine B (SRB) cytotoxicity assay; clonogenic cytotoxicity assay; glioma; brain tumor

1. Introduction

The vasculature associated with fast proliferating solid tumors is abnormal, which limits efficient oxygen supply and renders the tumor tissue hypoxic.¹⁻³ The presence of hypoxic areas in solid cancers has been correlated with resistance to chemo- and radiation therapies. Intratumoral hypoxia induces Hypoxia Inducible Factors (HIFs), transcription factors, which activate genes controlling mechanisms such as glycolysis, erythropoiesis, angiogenesis, and cell motility, which can benefit the survival of cancer cells.⁴⁻¹¹ HIFs can also influence the self-renewal of cancer stem cells (CSCs),¹²⁻¹⁴ and be activated in response to growth factors, oncogenes and inactivation of tumor suppressor genes.⁴⁻⁷

HIFs are heterodimeric protein complexes, composed of HIF- α and HIF- β subunits, which then associate with co-factors such as p300 and CBP to form active transcription factors. The regulation of HIFs largely occurs at the protein level, and is dependent upon the synthesis and stability of the HIF- α subunits. Under normoxia, HIF- α subunits are hydroxylated at proline residues by oxygen-dependent prolyl hydroxylases (PHDs), which mediates recognition by the Von Hippel-Lindau (VHL) E3 ubiquitin ligase complex and rapid degradation by the proteasome. Under hypoxia, HIF- α subunits are stabilized due to the inhibition of proline hydroxylation, and a functional HIF transcriptional complex is assembled, translocates to the nucleus and transcribes genes that contain DNA sequences called hypoxia response elements (HREs).⁴⁻⁷ Elevated levels of HIF-1 α have been correlated with poor prognosis of patients with glioblastoma (GBM), breast, pancreatic, colon and metastatic lung cancers.¹⁵⁻²⁴

Hypoxic tumor and HIF-1 have been evaluated as targets for anticancer therapy using a variety of approaches.²⁵⁻³⁰ While the differential function of HIF-1 and HIF-2 isoforms is still under investigation,⁷ both are associated with brain cancer stem cells,^{13, 14} and most studies suggest that one or both isoforms need targeting, depending on tumor and cancer type. Therefore, tumor cells over-expressing HIF represent an important target for anti-tumor therapy.³¹⁻³³ A number of existing chemotherapeutics can alter HIF activity as a result of their pleiotropic effects, including 2ME2, 17-DMAG, 17-AAG, camptothecin, PX-478, and YC-1.³⁴⁻⁴⁰ Most agents studied affect HIF indirectly via the inhibition of microtubule polymerization, Hsp90, topoisomerase I, thioredoxin 1, or other unknown mechanisms.^{41, 42} A search for more specific inhibitors used a screen targeting the interaction of HIF with the key transcriptional co-activator p300. Small molecule chetomin was identified, but later found to act as a general inhibitor of zinc ion binding proteins⁴³ and was abandoned due to unacceptable toxicity in mice.⁴⁴ A recent study suggests that acriflavine, an anti-trypanocidal, anti-bacterial and anti-viral agent interferes with HIF-1 α and β dimerization and possibly other signaling pathways such as NF- κ B.⁴⁵ It is too early to determine which agent affecting the HIF pathway will have the best anti-tumor efficacy and safety profile. It is also desirable to develop several agents that can interfere with HIF transcription in different ways so that we are prepared for the development of tumor resistance against single targeted sites.

We recently discovered 3,4-dimethoxy-*N*-[(2,2-dimethyl-2*H*-chromene-6-yl)methyl]-*N*-phenylbenzenesulfonamide (**1**)⁴⁶ as a novel small molecule HIF-1 pathway inhibitor in a high throughput screening among 10,000 molecular compounds that were based on a 2,2-dimethyl-2*H*-chromene structure as a naturally occurring biologically active lead moiety.^{40, 47-49}

Our ongoing investigations have identified the CH1 domain of p300/CBP as the putative target of **1**, and its binding is expected to disrupt the formation of the transcriptional complex among HIF-1 α , HIF-1 β , and p300/CBP under hypoxia.^{50, 51} **1** showed anticancer activity *in vivo* in brain, eye and pancreatic cancer models, however, it necessitated delivery in a formulation (Cremophor: ethanol= 1:1) due to poor aqueous solubility (0.009 μ M).^{52, 53} To develop analogs of **1** with improved aqueous solubility, we previously investigated structure activity relationships of fifteen lipophilic analogs and selected *N*-[(2,2-dimethyl-2*H*-chromene-6-yl)methyl]-*N*-(propan-2-yl)arylsulfonamides as the molecular motifs for further modifications described in the current study.⁵⁴

Here we designed *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]heteroarylsulfonamides (abbreviated hereafter as “heteroarylsulfonamides”) to possess molecular weights, estimated *logP* and *logS_w* values optimal for lead compounds in drug discovery.^{55, 56} Twelve heteroarylsulfonamides were synthesized, and their inhibitory potential against the transcriptional activity of HIF-1, effect on HIF-1 α synthesis and stability, physicochemical properties, metabolic stabilities, and cytotoxicities in human glioma and fibroblast cells were measured.

2. Results

2.1. Design of *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]heteroarylsulfonamides

The heteroarylsulfonamides were designed based on *N*-[(2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(propan-2-yl)arylsulfonamides (**2**, **3**, **4**, **5**, figure 1). Heteroarylsulfonyl groups were used in region 1 instead of arylsulfonyl groups to increase aqueous solubility. Small alkyl groups were used in region 2, to retain biological activity without abruptly increasing lipophilicity. The 2,2-dimethyl-2*H*-chromene structure in region 3 was diversified at the C-8 position by adding a hydroxyl or methoxy group due to synthetic feasibility. A hydroxyl group is advantageous in that it can be an anchor for functional groups to further increase diversity and structural flexibility of the compounds.

Twelve heteroarylsulfonamides (**6a** - **6l**) were selected for synthesis. (table 1) Their molecular weights range from 371 to 403 grams/mole, *logPs* from 3.4 to 4.2, and *logS_w*s from -4.2 to -3.2, which are optimal for lead compounds in drug discovery.^{55, 56}

2.2. Synthesis of *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]heteroarylsulfonamides

The chromene ring of 8-*R*-2,2-dimethyl-2*H*-chromene-6-carbaldehydes (**7a**, **7b**) was formed by Claisen cyclization of the propargyl ether, 3-*R*-4-[(2-methylbut-3-yn-2-yl)oxy]benzaldehyde, as described previously.^{54, 63} The *N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]alkylamines (**8a** - **8g**) were synthesized from **7a**, **7b** by acid-catalyzed imine formation, and then subsequent reduction with diisobutylaluminum hydride (DIBAL).

The final *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl] heteroarylsulfonamides (**6a** - **6g**, **6i** - **6k**) were synthesized from **8a** - **8g** and heteroarylsulfonyl chlorides. Several synthetic methods have been developed for the preparation of heteroarylsulfonyl chlorides, which are unstable molecules. These include low temperature oxidative chlorination of thio-

heterocycles with chlorine gas⁶⁴, chlorination of heteroaromatic methyl sulfides with sulfonyl chloride⁶⁵, oxidation of heteroaromatic thiols in a co-solvent of methylene chloride and 1 N aqueous hydrochloric acid containing 25 wt % calcium chloride with aqueous sodium hypochlorite⁶⁶, and oxidation of heteroaromatic thiols in concentrated sulfuric acid with aqueous sodium hypochlorite.^{67, 68} We synthesized heteroarylsulfonyl chlorides by the last method due to facile reaction conditions. Slow addition of aqueous sodium hypochlorite solution to the reaction mixture was crucial for high yield of the reaction, since low temperature has to be maintained during the highly exothermic reaction so as to prevent decomposition of the resulting heteroarylsulfonyl chlorides. The final heteroarylsulfonamides were formed in the presence of triethylamine at 40 °C (refluxing methylene chloride) in the case of pyridine-2-sulfonyl chloride, but, *N,N*-diisopropylethylamine (NIEA, Hünig's base) at 0 °C for the pyridine-4-sulfonyl, 1-methyl-1*H*-imidazol-2-sulfonyl, and 1,3-thiazol-2-sulfonyl chloride, since the latter heteroarylsulfonyl chlorides decomposed above 0 °C. For the reactions at 0 °C, the reaction mixture was gradually concentrated by argon gas to accelerate the reactions. (Scheme 1)

Insertion of a hydroxyl group at the C-8 position of the 2,2-dimethyl-2*H*-chromene ring was accomplished by chromenylation of 3,4-dihydroxybenzaldehyde with 3-methylbut-2-enal, for which the yield was low and not optimized.⁶⁹⁻⁷¹ The hydroxyl group at the C-8 position of the chromene ring was protected with methyl chloromethyl ether (MOMCl) to form a methoxymethyl ether, which was deprotected by 6 N aqueous HCl mixed with 1 equivalent of tetrahydrofuran (THF) at the end of the synthesis. (Scheme 2)

2.3. Inhibition of HIF transcriptional activity

The effects of twelve heteroarylsulfonamides on HIF transcriptional activity under hypoxia were measured by determining the luciferase activity present in LN229-V6R cells, which contain a stably integrated hypoxia/HIF inducible luciferase reporter gene.^{40, 46, 54} As a control for the absence of inhibition on luciferase enzyme *per se*, we also tested **1** and five heteroarylsulfonamides in LN229-Lux cells that contain a constitutive luciferase reporter gene driven by a retroviral LTR promoter. An Hsp90 inhibitor, 17-DMAG, and **1** were used as positive controls. 17-DMAG inhibited both reporter cell lines due to its known cytotoxicity; whereas **1** and heteroarylsulfonamides reduced luciferase activity only in LN229-V6R cells under hypoxia. The heteroarylsulfonamides decreased HIF-1 activity in a dose-dependent fashion with IC₅₀ values below (**6a**, **6b**, **6d**, **6e**, **6f**, **6h**, **6i**, **6l**) or close to 5 μM (**6c**, **6g**, **6j**, **6k**) (figure 2).

Region 1 of **6c**, **6i**, **6j**, and **6k** was diversified with pyridine-2-sulfonyl, pyridine-4-sulfonyl, 1-methyl-1*H*-imidazole-2-sulfonyl, and thiazole-2-sulfonyl groups, among which pyridine-4-sulfonyl group of **6i** showed the strongest HIF reporter inhibition. However, the pyridine-2-sulfonyl group of **6h** showed stronger inhibition than the pyridine-4-sulfonyl group of **6l**, when a hydroxyl group replaces the C-8 hydrogen in region 3.

Region 2 of **6a** ~ **6f** was varied with propan-2-yl, cyclopropyl, 2-methylpropan-1-yl, cyclopropylmethyl, cyclobutyl, and cyclopentyl group, among which cyclopropylmethyl of **6d** showed the strongest HIF reporter inhibition.

Region 3 of **6a** and **6g** were altered at the C-8 position of the 2,2-dimethyl-2*H*-chromene ring with a hydrogen and a methoxy group, and **6a** (hydrogen) showed the strongest inhibition. Region 3 of **6c** and **6i** were different at the same position with a hydrogen and a hydroxyl group. In this case, **6i** (hydroxyl) showed stronger inhibition at low concentration, however, dose-dependent inhibition increased more sharply for **6c** (hydrogen). Region 3 of **6i** and **6l** were modified in the same way as **6c** and **6g** with a hydrogen and a hydroxyl

group, of which **6l** (hydroxyl) showed slightly stronger inhibition at low concentration, however, dose-dependent inhibition increased more sharply for **6i** (hydrogen).

2.4. Inhibition of HIF-1 α Stability under Hypoxia

HIF-1 is a heterodimer of HIF-1 α and HIF-1 β subunits, which then associates with p300 or CBP cofactors to form an active transcriptional complex. We previously demonstrated that in contrast to many prior HIF inhibitors, **1** does not antagonize hypoxia-induced HIF-1 α expression under concentrations at which it blocks HIF transcription in the reporter assay.⁴³ To determine whether the heteroarylsulfonamides retain this property, we determined the levels of HIF-1 α protein under hypoxia in LN229-V6R cells. For these experiments we selected **6a**, **6g**, and **6l** because the three compounds exhibited different degrees of HRE reporter inhibition and possess structural diversities at the C-8 position of the chromene ring. We investigated if the levels of HIF-1 α protein would correlate with transcriptional inhibition of the HRE reporter by the compounds. LN229-V6R cells were treated with the compounds at concentrations of 3, 6, 12, 25, 50, and 100 μ M under hypoxia for 24 hours, and then HIF-1 α protein levels in the cell extracts were examined by western blotting. **6a** had no effect on HIF-1 α levels at all concentrations tested, except for a slight reduction at the highest concentration (100 μ M), likely due to non-specific toxicity. **6g** and **6l** decreased HIF-1 α levels in a dose-dependent fashion starting from 12 and 25 μ M respectively (figure 3). In comparison, in the previous HRE-mediated luciferase assays HIF-1 transcriptional activity was inhibited completely by **6a**, and by more than 60 ~ 80 % by **6g**, and **6l** when tested at 10 μ M. These results indicate that **6a**, **6g** and **6l** most likely inhibit HIF transcription in a protein stability independent way similar to **1**. The reduction in HIF-1 α levels afforded by all 3 compounds at 100 μ M may reflect non-specific cytotoxicity.

2.5. Physicochemical Property and Metabolic Stability

Experimental determinations of $LogP_{7.4}$, aqueous solubility, and metabolic stability of a selected number of the heteroarylsulfonamides were performed to further characterize the compounds. (Table 2)

$LogP_{7.4}$ was measured by either the shake flask method (**6a**, **6g**) or the HPLC method (**1**, **6b** ~ **6f**, **6h** ~ **6l**) according to OECD guidelines.⁷² Most compounds followed the trends predicted by *in silico* calculations and the measured values were smaller than the predicted values by 1.0 ~ 1.5. When a methoxy group or hydroxyl group is placed at the C-8 position of the chromene ring instead of a hydrogen as in **6g**, **6h**, and **6l**, the $LogP_{7.4}$ value decreased by more than 2 from the predicted values to result in 1.2 ~ 1.3. The presence of a 2-methylpropan-2-yl group in region 2 conferred high $LogP_{7.4}$ values and heteroarylsulfonyl groups in region 1 also affected $LogP_{7.4}$ values as shown for **6c**, **6i**, **6j**, and **6k**.

Aqueous solubilities of **1**, **6a**, **6d**, and **6g** were quantified by HPLC coupled with a UV detector⁶⁷ on saturated aqueous suspensions after filtration with a polytetrafluoroethylene (PTFE) filter (pore size: 0.2 μ m). **6a** and **6d** were chosen due to their strong activity in the HRE-reporter assay, and **6g** to evaluate the influence of the methoxy group attached to the C-8 position of the chromene ring. Additional measurements of aqueous solubility of **1** and **6a** were performed by laser nephelometry at three different pHs (3.0, 5.0, and 7.4). Aqueous solubilities of **6a**, **6d**, and **6g** are, respectively, 100, 20, and 9,000 times better than **1**, which are consistent with the $logS_w$ predictions for **6a** and **6d**. The substitution of hydrogen with a methoxy group at the C-8 position of the chromene ring increased aqueous solubility by 90 times (compare **6a** and **6g**). pH did not affect aqueous solubility of **1**, however, low pH increased aqueous solubility of **6a** by 50 - 60 times (pH 5.0, pH 3.0) due to the presence of the basic nitrogen in the pyridin-2-sulfonyl group in region 1.

Metabolic stabilities of **1**, **6a**, **6d**, **6g**, and **6l** were measured in mouse plasma and homogenates of mouse liver in PBS (1:2 (w/v)). The concentrations of all compounds did not decrease by more than 1 % when the compounds were incubated in mouse plasma at 37 °C for 24 hours, which indicated absence of degradation or metabolism in plasma. All compounds underwent *ex-vivo* hepatic metabolism with half-lives shown in table 2, in which **1** showed the fastest and **6a** the slowest metabolism. (See a graph in the supporting information.)

2.6. Inhibition of Cell Viability/Proliferation

To determine whether **6a**, **6g**, and **6l** altered tumor cell growth in culture, we performed sulforhodamine B (SRB) assays in LN229-V6R glioma cells in 3 days, and to further examine the cell growth inhibitory activity of **6a**, **6g**, and **6l** in an independent biological assay, we performed clonogenicity assays in LN229 human glioblastoma cells and HFF-1 immortalized human fibroblasts, over a period of 14 days. IC_{50} values (μ M) of SRB and clonogenicity assays were presented in table 3.

2.6.1. Sulforhodamine B (SRB) Assay⁶⁶—LN229-V6R cells (5×10^3 cells/well) were seeded and cultured under normoxia for 24 hours, and cell densities were measured. The cells were then treated with serially diluted **6a**, **6g**, and **6l** (1.56 - 100 μ M; 1% DMSO final in culture medium) under normoxia or hypoxia for 72 hours. The three heteroarylsulfonamides showed stronger cytotoxicity under normoxia than under hypoxia. The IC_{50} values of cytotoxicity of the three heteroarylsulfonamides under hypoxia was over 100 μ M in three days, whereas all of them decreased HRE-reporter activity by 60 ~ 95 % at 10 μ M in one day.

2.6.2. Clonogenicity Assays—**6a**, **6g**, and **6l** showed stronger inhibition of cell proliferation under normoxia than under hypoxia in both of glioblastoma and fibroblast cell lines, which was the similar result as those of SRB assays. The cytotoxicity profiles of each compound against glioblastoma, LN229, and fibroblast, HFF-1, cell lines were similar, suggesting nonspecific cytotoxicities. **6l** displayed the strongest cytotoxicity in both of the two cell lines.

3. Discussion and Conclusions

We previously discovered **1** as a potent HIF-1 pathway inhibitor with a cell-based high-throughput assay.⁴⁶ **1** contains a 2,2-dimethyl-2*H*-chromene ring found in many natural products. For example, 2,2-dimethyl-2*H*-chromene based molecules isolated from *Blepharispermum subsessile*, and the leaves of *Piper aduncum* L. have antifungal, antibacterial, and trypanocidal activities.⁷³⁻⁷⁵ The chromenes extracted from the leaves of *Melicope lunu-ankenda* have antipyretic, analgesic, anti-inflammatory, and antioxidant activities.⁷⁶ Further structure-activity relationship (SAR) studies showed that *N*[(2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(propan-2-yl)arylsulfonamides possess strong HIF-1 pathway inhibition and are amenable for further chemical optimization.⁵⁴

Here, we improved the prior compounds by designing a series of 2,2-dimethyl-2*H*-chromene based heteroarylsulfonamides with the purpose of decreasing lipophilicity and increasing aqueous solubility. We modified the *N*[(2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(propan-2-yl)arylsulfonamides, by substituting the arylsulfonyl group in region 1 with heteroarylsulfonyl groups such as pyridine-2-sulfonyl, pyridine-4-sulfonyl, 1-methyl-1*H*-imidazole-2-sulfonyl, and thiazole-2-sulfonyl groups, by replacing the propan-2-yl group in region 2 with short alkyl groups that mainly consist of small cyclic alkyl groups, and by derivatizing the chromene moiety at the C-8 position in region 3.

Twelve heteroarylsulfonamides were designed to possess optimal $\log P$ and $\log S_w$ values by *in silico* calculations and subsequently synthesized. All twelve heteroarylsulfonamides show inhibition of HIF-1 transcription in a reporter assay at low micromolar concentrations, and the mechanism of action appeared independent of alteration in HIF-1 α protein expression. Our ongoing studies suggest that the mechanism of action of **1** involves the disruption of the interaction between HIF-1 α and its co-factors p300/CBP by antagonizing the CH1 domain of p300.^{50, 51} We expect that heteroarylsulfonamides will possess similar biological activities, however, this will need further experimental validation.

Eight heteroarylsulfonamides showed lower $\log P_{7.4}$ values ranging from 1.2 to 3.1 *versus* 3.7 for **1**. Increased aqueous solubilities were achieved for compounds **6a**, **6d**, and **6g**, which showed 100, 20, and 9000 times more soluble than **1**, respectively. The stabilities of **6a**, **6d**, **6g**, and **6l** in mouse plasma were similar to the parent compound **1**, however, they exhibited longer half-lives in homogenized mouse liver ranging from 13 -20 hrs, compared with 11 hours for **1**, which suggests that they will likely be more resistant to *in vivo* degradation and metabolism than **1**.

In conclusion, the novel heteroarylsulfonamides we synthesized have dramatically improved aqueous solubility, less lipophilicity, and slightly better stability, while retaining much of the bioactivity of the parent compound.

We developed novel heteroarylsulfonamides to optimize pharmacological properties of **1**, and investigated their structure-activity relationships to improve HIF-1 inhibitory activity simultaneously. Six-membered heteroaryl groups in region 1, a propan-2-amine in region 2, and a (6-hydroxy-2,2-dimethyl-2H-chromen-6-yl)methyl group in region 3, were the best selections to increase aqueous solubility and HIF-1 inhibition, and decrease lipophilicity. 1-Cyclopropylmethanamine and cyclobutanamine in region 2 showed better HIF-1 inhibition than propan-2-amine; however, lipophilicity, aqueous solubility, and metabolic stability made the latter group as the superior choice. A functional group at the C-8 of the chromene ring significantly affected physicochemical properties and HIF-1 inhibition, so a further investigation of derivatization is required.

The next step towards the clinical translation of the heteroarylsulfonamides will be to optimize their pharmacological properties *in vivo* and test their anti-tumor properties in animal models for cancer.

4. Experimental section

4.1. Synthesis

Reagents were purchased from Sigma-Aldrich, Alfa Aesar and Fisher Scientific. Anhydrous solvents were purchased from Sigma-Aldrich. Glassware was oven-dried at 200 °C and cooled under argon to room temperature. All reactions were performed under argon gas. A column chromatography was performed with Silica Gel 60 (Particle Size: 40 - 63 μ m) with less than 4 psi of argon pressure.

Thin layer chromatography was performed with TLC Silica gel 60 F₂₅₄ and organic compounds were visualized by UV light (254 nm), iodine vapor, phosphomolybdic acid (10 % (w/v) in ethanol) staining with heating, and ninhydrin solution with heating for amines. NMR spectroscopy was recorded by Varian INOVA 400, Mercury 300 and VNMRs 400 spectrometers. Mass spectroscopy was obtained by JEOL JMS-SX102/SX102A/E. HPLC was measured by Waters 1525 Binary HPLC pump coupled with a Waters 2487 Dual λ Absorbance Detector, and data was processed by Waters Breeze GPC Software. Dual wavelengths of 254 and 280 nm were used for UV absorbance measurements. The running

time of HPLC for each compound was 20 minutes and the peaks were integrated after 3.5 minute.

The purity of **6a** – **6l** was over 95%, which was determined by % area of its UV absorbance peak at 254 nm by two different HPLC systems. In the first HPLC system, a symmetry C₁₈ column, WAT045905 (5 μ m, 4.6 $\times$ 150 mm), was used with an eluent (70: 30: 0.1 = methanol: water: triethylamine) at a flow rate of 2 mL/min. In the second HPLC system, a Nova-Pak C₁₈ column, WAT011695 (4 μ m, 3.9 $\times$ 300 mm), was used with an eluent (60: 40: 0.1 = 90 % ethanol, 5 % methanol, 5 % isopropanol: water: triethylamine) at a flow rate at 0.8 mL/minute. An alcohol mix of 90 % of ethanol, 5 % methanol and 5 % isopropanol was used instead of pure ethanol due to an institutional regulation limiting ethanol purchase.

2,2-dimethyl-2H-chromene-6-carbaldehyde or 8-methoxy-2,2-dimethyl-2H-chromene-6-carbaldehyde (7a, 7b)—An aqueous sodium hydroxide solution (4 N, 5 mL) was added to a solution of 4-hydroxybenzaldehyde (2.38 g, 19.5 mmol) and 3-chloro-3-methyl-1-butyne (1 g, 9.75 mmol) in 8 mL of dimethylformamide (DMF), to form a binary solution. The reaction mixture was stirred vigorously at 60 °C overnight. After cooling, 20 mL of water was added to the reaction mixture, which was extracted with diethyl ether (30 mL $\times$ 3). The combined organic phase was washed with 40 mL of 1 N aqueous sodium hydroxide and then brine, dried with anhydrous magnesium sulfate, and concentrated *in vacuo*. Formation of crude 4-[(2-methylbut-3-yn-2-yl)oxy]benzaldehyde was confirmed by TLC with ethyl acetate/hexane (1/8) and NMR. ¹H NMR (CDCl₃): δ 1.73 (s, 6H), 2.67 (s, 1H), 7.34 (d, *J* = 8.8 Hz, 1H), 7.83 (d, *J* = 8.8 Hz, 1H), 9.91 (s, 1H).

Crude 4-[(2-methylbut-3-yn-2-yl)oxy]benzaldehyde was dissolved in 10 mL of *N*-methylpyrrolidone (NMP, bp = 202 °C - 204 °C) and refluxed overnight. After cooling to room temperature, 80 mL of water was added to the solution, which was extracted with diethyl ether (100 mL $\times$ 3). The combined organic phase was washed with brine, dried with anhydrous magnesium sulfate and concentrated *in vacuo*. **7a** was purified by silica gel chromatography with ethyl acetate/hexane (1/8) as a viscous yellowish liquid. (565 mg, 15 % yield) ¹H NMR (CDCl₃): δ 1.46 (s, 6H), 5.68 (d, *J* = 9.9 Hz, 1H), 6.36 (d, *J* = 9.9 Hz, 1H), 6.85 (d, *J* = 8.05 Hz, 1H), 7.50 (d, *J* = 2.37 Hz, 1H), 7.63 (dd, *J* = 8.52, 1.89 Hz, 1H), 9.83 (s, 1H).

HRMS *m/z* M⁺ calcd 189.09101, found 189.09071.

7b was synthesized by the same method as that of **7a**. Vanillin (3 g, 20 mmol) and 3-chloro-3-methyl-1-butyne (1 g, 10 mmol) were used as starting materials. **7b** was purified by silica gel chromatography with ethyl acetate/hexane (1/6) as a white solid. (204 mg, 5 % in two steps)

3-Methoxy-4-[(2-methylbut-3-yn-2-yl)oxy]benzaldehyde—¹H NMR (CDCl₃): δ 1.75 (s, 6H), 2.63 (s, 1H), 3.90 (s, 3H), 7.43~7.40 (m, 2H), 7.64 (d, *J* = 8.79 Hz, 1H).

8-Methoxy-2,2-dimethyl-2H-chromene-6-carbaldehyde (7b)—¹H NMR (CDCl₃): δ 1.51 (s, 6H), 3.93 (s, 3H), 5.70 (d, *J* = 9.78 Hz, 1H), 6.37 (d, *J* = 9.78 Hz, 1H), 7.17 (d, *J* = 1.96 Hz, 1H), 7.32 (d, *J* = 1.96 Hz, 1H), 9.81 (s, 1H). HRMS *m/z* M⁺ calcd 219.10157, found 219.10124.

N-[(2,2-dimethyl-2H-chromen-6-yl)methyl]propan-2-amine (8a)—**7a** (102 mg, 0.54 mmol) was dissolved in 2 mL of anhydrous methylene chloride, to which *p*-toluenesulfonic acid monohydrate (20 mg, 0.1 mmol) and propan-2-amine (1 mL, 12 mmol) were added. The reaction mixture was refluxed overnight and then concentrated *in vacuo*. The NMR of

the crude product confirmed a complete conversion of the aldehyde, **7a**, to the corresponding imine by disappearance of the aldehyde proton peak, 9.83 ppm (s, 1H) and appearance of the imine proton peak, 8.19 ppm (s, 1H).

The crude imine was suspended in 3 mL of toluene, to which 1 M diisobutylaluminum hydride in toluene (DIBAL, 2.5 mL, 2.5 mmol) was added slowly to control vigorous bubbling. The reaction mixture was stirred overnight at room temperature, and then 20 mL of 1 N aqueous hydrochloric acid was added to the reaction mixture slowly, to quench the reduction. The resulting emulsion was basified with concentrated aqueous sodium carbonate solution. The reaction mixture was extracted with ethyl acetate (20 mL $\times$ 3). The combined organic phase was washed with brine, dried with anhydrous magnesium sulfate, and then concentrated *in vacuo*. **8a** was purified by silica gel chromatography with triethylamine/methanol/methylene chloride (1/1/100) as a viscous oil. (111 mg, 89 % in two steps.)

^1H NMR (CDCl_3): δ 1.1 (d, J = 6.15 Hz, 6H), 1.4 (s, 6H), 2.85 (septet, J = 6.45 Hz, 1H), 3.66 (s, 2H), 5.6 (d, J = 9.96 Hz, 1H), 6.3 (d, J = 9.67 Hz, 1H), 6.72 (d, J = 8.2 Hz, 1H), 6.95 (d, J = 2.05 Hz, 1H), 7.04 (dd, J = 2.34, 7.91 Hz, 1H).

Other amines (**8b**, **8c**, **8d**, **8e**, **8f**, and **8g**) were synthesized by the same method as that of **8a**. The imine formation was confirmed by disappearance of the aldehyde proton peak (9.83 ppm (**7a**) or 9.81 ppm (**7b**)) and appearance of the corresponding imine peak (8.1 ppm (s, 1H) - 8.3 ppm (s, 1H)). The amines were synthesized by reduction of the corresponding imines.

***N*-(2,2-dimethyl-2*H*-chromen-6-yl)methyl]cyclopropanamine (8b)**—Yield = 37 % in two steps

^1H NMR (CDCl_3): δ 0.93 (t, J = 7.33 Hz, 2H), 1.43 (s, 6H), 1.56 (sextet, J = 7.33 Hz, 1H), 2.61 (t, J = 7.33 Hz, 2H), 3.70 (s, 2H), 5.61 (d, J = 9.67 Hz, 1H), 6.31 (d, J = 9.67 Hz, 1H), 6.73 (d, J = 8.20 Hz, 1H), 6.97 (d, J = 2.05 Hz, 1H), 7.06 (dd, J = 2.05, 8.20 Hz, 1H).

***N*-(2,2-dimethyl-2*H*-chromen-6-yl)methyl]-2-methylpropan-1-amine (8c)**—Yield = 30 % in two steps.

^1H NMR (CDCl_3): δ 0.92 (d, J = 6.45 Hz, 6H), 1.43 (s, 6H), 1.79 (septet, J = 6.45, 6.74 Hz, 1H), 2.44 (d, J = 7.03 Hz, 2H), 3.68 (s, 2H), 5.61 (d, J = 9.96 Hz, 1H), 6.32 (d, J = 9.96 Hz, 1H), 6.73 (d, J = 8.20 Hz, 1H), 6.96 (d, J = 2.05 Hz, 1H), 7.05 (dd, J = 2.05, 7.91 Hz, 1H).

1-Cyclopropyl-*N*-(2,2-dimethyl-2*H*-chromen-6-yl)methyl]methamine (8d)—Yield = 77 % in two steps.

^1H NMR (CDCl_3): δ 0.13 (m, 2H), 0.47~0.53 (m, 2H), 0.97~1.05 (m, 1H), 1.42 (s, 6H), 2.51 (d, J = 6.74 Hz, 2H), 3.73 (s, 2H), 5.61 (d, J = 9.67 Hz, 1H), 6.32 (d, J = 9.67 Hz, 1H), 6.73 (d, J = 8.20 Hz, 1H), 6.98 (d, J = 2.05 Hz, 1H), 7.07 (dd, J = 2.05, 8.20 Hz, 1H).

***N*-(2,2-dimethyl-2*H*-chromen-6-yl)methyl]cyclobutanamine (8e)**—Yield = 60 % in two steps.

^1H NMR (CDCl_3): δ 1.42 (s, 6H), 1.59~1.75 (m, 5H), 2.19~2.27 (m, 2H), 3.25~3.35 (m, 1H), 3.60 (s, 2H), 5.60 (d, J = 9.67 Hz, 1H), 6.31 (d, J = 9.67 Hz, 1H), 6.72 (d, J = 8.20 Hz, 1H), 6.95 (d, J = 2.05 Hz, 1H), 7.04 (dd, J = 2.05, 8.20 Hz, 1H).

N-[(2,2-dimethyl-2H-chromen-6-yl)methyl]cyclopentanamine (8f)—Yield = 67 % in two steps.

¹H NMR (CDCl₃): δ 1.36~1.42 (m, 3H), 1.42 (s, 6H), 1.51~1.56 (m, 2H), 1.68~1.73 (m, 2H), 1.84~1.89 (m, 2H), 3.12 (quintet, *J* = 6.74 Hz, 1H), 3.67 (s, 2H), 5.60 (d, *J* = 9.67 Hz, 1H), 6.31 (d, *J* = 9.96 Hz, 1H), 6.72 (d, *J* = 8.20 Hz, 1H), 6.96 (d, *J* = 2.05 Hz, 1H), 7.05 (dd, *J* = 2.05, 8.20 Hz, 1H).

N-[8-methoxy-(2,2-dimethyl-2H-chromen-6-yl)methyl]propan-2-amine (8g)—Yield = 39 % in two steps.

¹H NMR (CDCl₃): δ 1.11 (d, *J* = 6.15 Hz, 6H), 1.46 (s, 6H), 2.88 (septet, *J* = 6.15 Hz, 1H), 3.68 (s, 2H), 3.86 (s, 3H), 5.61 (d, *J* = 9.67 Hz, 1H), 6.28 (d, *J* = 9.96 Hz, 1H), 6.60 (s, 1H), 6.78 (s, 1H).

N-[(2,2-dimethyl-2H-chromen-6-yl)methyl]-N-(propan-2-yl)pyridine-2-sulfonamide (6a)

Pyridine-2-sulfonyl chloride: 2-Mercaptopyridine (126 mg, 1 mmol) was dissolved in 5 mL of *conc.* sulfuric acid to form a yellow solution, which was cooled to around -15 °C with sodium chloride/ice (1/3) bath. 10 - 15 % aqueous sodium hypochlorite solution (11 mL, 15 - 20 mmol) was added to the solution slowly enough to maintain the internal temperature of the reaction mixture below 10 °C, with vigorous stirring. The reaction mixture was stirred at 0 °C for one hour and then 10 mL of water was added, which was then extracted with methylene chloride (20 mL × 3). The combined organic phase was washed with water, dried with anhydrous magnesium sulfate, and then concentrated *in vacuo*. Pyridine-2-sulfonyl chloride (145 mg, 72 %) was produced as a yellowish viscous liquid. ¹H NMR (CDCl₃): δ 7.684~7.729 (m, 1H), 8.043~8.143 (m, 2H), 8.84 (d, *J* = 4.10 Hz, 1H).

Freshly prepared pyridine-2-sulfonyl chloride (100 mg, 0.6 mmol) was added to a solution of **8a** (25 mg, 0.1 mmol) and triethylamine (0.5 mL, 3.6 mmol) in 0.8 mL of methylene chloride. The reaction mixture was refluxed overnight and then concentrated *in vacuo*. **6a** (31 mg, 77 %) was purified by silica gel chromatography with ethyl acetate/hexane (1/1).

¹H NMR (CDCl₃): δ 1.03 (d, *J* = 6.74 Hz, 6H), 1.42 (s, 6H), 4.27 (septet, *J* = 6.74 Hz, 1H), 4.45 (s, 2H), 5.61 (d, *J* = 9.67 Hz, 1H), 6.30 (d, *J* = 9.67 Hz, 1H), 6.68 (d, *J* = 8.21 Hz, 1H), 7.04 (d, *J* = 2.05 Hz, 1H), 7.10 (dd, *J* = 2.05, 8.20 Hz, 1H), 7.38 - 7.47 (m, 1H), 7.81 - 7.96 (m, 2H), 8.7 (d, *J* = 4.40 Hz, 1H).

HRMS *m/z* [M+Na]⁺ calcd 395.13999, found 395.14017.

HPLC-1: *t*_R = 4.5 minute, Purity = 98 %.

HPLC-2: *t*_R = 6.5 minute, Purity = 99 %.

Other heteroarylsulfonamides containing a pyridine-2-sulfonyl group were synthesized by the same method as that of **6a**.

N-cyclopropyl-N-[(2,2-dimethyl-2H-chromen-6-yl)methyl]pyridine-2-sulfonamide (6b)—Purification: silica gel chromatography with ethyl acetate/hexane (2/3); Yield = 24 %. ¹H NMR (CDCl₃): δ 0.725 (t, *J* = 7.33 Hz, 2H), 1.35~1.43 (m, 1H), 3.23 (dd, *J* = small as broadening of peaks, 7.62 Hz, 2H), 4.44 (s, 2H), 5.61 (d, *J* = 9.96 Hz, 1H), 6.27 (d, *J* = 9.97 Hz, 1H), 6.68 (d, *J* = 8.20 Hz, 1H), 6.93 (d, *J* = 1.76 Hz, 1H), 7.01 (dd,

$J = 2.05, 8.20$ Hz, 1H), 7.47 (ddd, $J = 0.88, 4.69, 7.32$ Hz, 1H), 7.85 - 7.99 (m, 2H), 8.70 (d, $J = 4.69$ Hz, 1H).

HRMS m/z [M+Na]⁺ calcd 393.12488, found 395.14008.

HPLC-1: $t_R = 5.1$ minute, Purity = 100 %.

HPLC-2: $t_R = 6.8$ minute, Purity = 97 %.

***N*-(2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(2-methylpropyl)pyridine-2-sulfonamide (6c)**—Purification: silica gel chromatography with ethyl acetate/hexane (1/4); Yield = 60 %. ¹H NMR (CDCl₃): δ 0.76 (d, $J = 6.74$ Hz, 6H), 1.42 (s, 6H), 1.74 (septet, $J = 7.03$ Hz, 1H), 3.11 (d, $J = 7.325$ Hz, 2H), 4.42 (s, 2H), 5.60 (d, $J = 9.67$ Hz, 1H), 6.24 (d, $J = 9.67$ Hz, 1H), 6.65 (d, $J = 8.20$ Hz, 1H), 6.87 (d, $J = 2.05$ Hz, 1H), 6.96 (dd, $J = 2.05, 8.20$ Hz, 1H), 7.45 (ddd, $J = 1.17, 4.69, 7.62$ Hz, 1H), 7.865 (td, $J = 1.76, 7.62$ Hz, 1H), 7.95 (d, $J = 7.62$ Hz, 1H), 8.69 (d, $J = 4.69$ Hz, 1H).

HRMS m/z [M+Na]⁺ calcd 409.15564, found 409.15649.

HPLC-1: $t_R = 6.8$ minute, Purity = 100 %.

HPLC-2: $t_R = 8.4$ minute, Purity = 97 %.

***N*-(cyclopropylmethyl)-*N*-(2,2-dimethyl-2*H*-chromen-6-yl)methyl]pyridine-2-sulfonamide (6d)**—Purification: silica gel chromatography with ethyl acetate/hexane (1/2); Yield = 24 %. ¹H NMR (CDCl₃): δ 0.0115 (d, $J = 5.27$ Hz, 2H), 0.301 (d, $J = 7.62$ Hz, 2H), 0.67~0.825 (m, 1H), 1.425 (s, 6H), 3.17 (d, $J = 6.74$ Hz, 2H), 4.58 (s, 2H), 5.61 (d, $J = 9.67$ Hz, 1H), 6.28 (d, $J = 9.67$ Hz, 1H), 6.69 (d, $J = 8.21$ Hz, 1H), 6.975 (s, 1H), 7.04 (d, $J = 7.91$ Hz, 1H), 7.47 (dd, $J = 4.98, 7.62$ Hz, 1H), 7.88 (t, $J = 7.62$ Hz, 1H), 7.99 (d, $J = 7.62$ Hz, 1H), 8.71 (d, $J = 4.69$ Hz, 1H).

HRMS m/z [M+Na]⁺ calcd 407.13999, found 407.14046.

HPLC-1: $t_R = 5.7$ minute, Purity = 100 %.

HPLC-2: $t_R = 7.5$ minute, Purity = 95 %.

***N*-(cyclobutyl)-*N*-(2,2-dimethyl-2*H*-chromen-6-yl)methyl]pyridine-2-sulfonamide (6e)**—Purification: silica gel chromatography with ethyl acetate/hexane (1/2); Yield = 65%. ¹H NMR (CDCl₃): δ 1.42 (s, 6H), 1.42~2.04 (m, 6H), 4.31~4.39 (m, 1H), 4.51 (s, 2H), 5.61 (d, $J = 9.96$ Hz, 1H), 6.30 (d, $J = 9.67$ Hz, 1H), 6.69 (d, $J = 8.20$ Hz, 1H), 7.00 (s, 1H), 7.06 (dd, $J = 2.05, 8.205$ Hz, 1H), 7.43 - 7.48 (m, 1H), 7.82 - 7.94 (m, 2H), 8.69 (d, $J = 3.81$ Hz, 1H).

HRMS m/z [M+Na]⁺ calcd 407.13999, found 407.14034.

HPLC-1: $t_R = 5.6$ minute, Purity = 100 %.

HPLC-2: $t_R = 7.1$ minute, Purity = 96 %.

***N*-(cyclopentyl)-*N*-(2,2-dimethyl-2*H*-chromen-6-yl)methyl]pyridine-2-sulfonamide (6f)**—Purification: silica gel chromatography with ethyl acetate/hexane (1/4); Yield = 16 %. ¹H NMR (CDCl₃): δ 1.26~1.59 (m, 8H), 1.43 (s, 6H), 4.31~4.40 (m, 1H), 4.48 (s, 2H), 5.61 (d, $J = 9.67$ Hz, 1H), 6.30 (d, $J = 9.67$ Hz, 1H), 6.69 (d, $J = 8.21$ Hz,

1H), 7.03 (s, 1H), 7.07 (d, J = 8.50 Hz, 1H), 7.44 - 7.48 (m, 1H), 7.83 - 7.94 (m, 2H), 8.71 (d, J = 4.69 Hz, 1H).

HRMS m/z [M+Na]⁺ calcd 421.15564, found 421.15577.

HPLC-1: t_R = 7.3 minute, Purity = 100 %.

HPLC-2: t_R = 8.4 minute, Purity = 96 %.

***N*-[8-methoxy-2,2-dimethyl-2*H*-chromen-6-yl)methyl]pyridine-2-sulfonamide (6g)**—Purification: silica gel chromatography with ethyl acetate/hexane (2/1); Yield = 23 %. ¹H NMR (CDCl₃): δ 1.05 (d, J = 6.74 Hz, 6H), 1.47 (s, 6H), 3.84 (s, 3H), 4.27 (septet, J = 6.74 Hz, 1H), 4.45 (s, 2H), 5.61 (d, J = 9.96 Hz, 1H), 6.27 (d, J = 9.96 Hz, 1H), 6.62 (s, 1H), 6.88 (s, 1H), 7.43 - 7.48 (m, 1H), 7.82~7.93 (m, 2H), 8.70 (d, J = 4.40 Hz, 1H).

HRMS m/z [M+Na]⁺ calcd 425.15055, found 425.15089.

HPLC-1: Flow rate: 2 mL/minute: t_R = 2.9 minute, Purity = 97 %; Flow rate: 1 mL/minute: t_R = 5.6 minute, Purity = 99 %

HPLC-2: t_R = 5.1 minute, Purity = 100 %.

***N*-[2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(2-methylpropyl)pyridine-4-sulfonamide (6i)**

Pyridine-4-sulfonyl chloride: Pyridine-4-sulfonyl, 1,3-thiazole-2-sulfonyl, and 1-methyl-1*H*-imidazole-2-sulfonyl chloride were synthesized by the same method as that of pyridine-2-sulfonyl chloride. They were used immediately after synthesis due to their instabilities at temperatures above 0 °C. Ten theoretical equivalents of the sulfonyl chloride were synthesized, which were extracted with 10 mL of cold methylene chloride, washed with cold brine and then dried with anhydrous magnesium sulfate. The sulfonyl chloride solution in methylene chloride was immersed in ice-water bath and concentrated with argon flow until the volume was reduced to 3 mL.

8a (25 mg, 0.1 mmol) was dissolved in 0.8 mL of methylene chloride, to which 0.5 mL of *N,N*-diisopropylethylamine (DIEA, 0.5 mL, 3 mmol) was added and cooled to 0 °C. Ten equivalents of freshly prepared pyridine-4-sulfonyl chloride were added to the reaction mixture slowly, which was stirred at 0 °C for 3 hours with slow argon flow. **6i** (25 mg, 63 %) was purified by silica gel chromatography with ethyl acetate/hexane (1/5). ¹H NMR (CDCl₃): δ 0.78 (d, J = 6.74 Hz, 6H), 1.43 (s, 6H), 1.72~1.82 (m, 1H), 2.96 (d, J = 7.62 Hz, 2H), 4.27 (s, 2H), 5.62 (d, J = 9.96 Hz, 1H), 6.21 (d, J = 9.96 Hz, 1H), 6.68 (d, J = 7.91 Hz, 1H), 6.77 (d, J = 2.05 Hz, 1H), 6.91 (dd, J = 2.05, 8.50 Hz, 1H), 7.63 (d, J = 5.86 Hz, 1H), 8.82 (d, J = 6.15 Hz, 1H).

HRMS m/z [M+Na]⁺ calcd 386.16641, found 387.17447.

HPLC-1: t_R = 6.5 minute, Purity = 98 %.

HPLC-2: t_R = 8.4 minute, Purity = 96 %.

6j and **6k** were synthesized by the same method as that of **6i**.

***N*-[2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(2-methylpropyl)-1-methyl-1*H*-imidazole-2-sulfonamide (6j)**—Purification: silica gel chromatography with ethyl acetate/hexane (1/2), Yield = 58 %. ¹H NMR (CDCl₃): δ 0.77 (d, J = 6.45 Hz, 6H), 1.43 (s,

6H), 1.63~1.70 (m, 1H), 3.20 (d, J = 7.62 Hz, 1H), 3.90 (s, 3H), 4.47 (s, 2H), 5.62 (d, J = 9.96 Hz, 1H), 6.29 (d, J = 9.67 Hz, 1H), 6.71 (d, J = 8.20 Hz, 1H), 6.93~6.94 (m, 2H), 7.04 (dd, J = 2.34, 8.20 Hz, 1H), 7.08 (d, J = 0.88 Hz, 1H).

HRMS m/z [M+Na]⁺ calcd 412.16654, found 412.16699.

HPLC-1: t_R = 5.8 minute, Purity = 100 %.

HPLC-2: t_R = 8.7 minute, Purity = 97 %.

***N*-(2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(2-methylpropyl)-1,3-thiazole-2-sulfonamide (6k)**—Purification: silica gel chromatography with ethyl acetate/hexane (1/2), Yield = 17 %. ¹H NMR (CDCl₃): δ 0.79 (d, J = 6.45 Hz, 6H), 1.42 (s, 6H), 1.77~1.81 (m, 1H), 3.12 (d, J = 7.62 Hz, 2H), 4.43 (s, 2H), 5.62 (d, J = 9.96 Hz, 1H), 6.26 (d, J = 9.67 Hz, 1H), 6.68 (d, J = 8.20 Hz, 1H), 6.87 (d, J = 2.34 Hz, 1H), 6.97 (dd, J = 2.34, 8.20 Hz, 1H), 7.58 (d, J = 3.22 Hz, 1H), 7.95 (d, J = 3.22 Hz, 1H).

HRMS m/z [M+Na]⁺ calcd 415.11206, found 415.11253.

HPLC-1: t_R = 8.0 minute, Purity = 100 %.

HPLC-2: t_R = 10 minute, Purity = 89 %.

8-Hydroxy-2,2-dimethyl-2*H*-chromene-6-carbaldehyde (9)—3,4-

Dihydroxybenzaldehyde (2.3 g, 17 mmol) and 3-methylbut-2-enal (1 mL, 10 mmol) were dissolved in 3 mL of pyridine, and refluxed overnight. Pyridine was evaporated under vacuum, and the crude reaction mixture was dissolved in methylene chloride. **9** (103 mg, 2.4%) was purified with silica gel chromatography with ethyl acetate/hexane (1/50). ¹H NMR (CDCl₃): δ 1.50 (s, 6H), 5.70 (d, J = 10.1 Hz, 1H), 6.38 (d, J = 10.1 Hz, 1H), 7.15 (d, J = 2.14 Hz, 1H), 7.32 (d, J = 1.83 Hz, 1H), 9.78 (s, 1H).

8-(Methoxymethoxy)-2,2-Dimethyl-2*H*-chromene-6-carbaldehyde (10)—

Chloromethyl methyl ether (4 mL, 5 mmol) was added to a solution of **9** (103 mg, 0.5 mmol) and *N,N*-diisopropylethylamine (0.2 mL, 1 mmol) in 4 mL of methylene chloride. The reaction mixture was refluxed overnight and concentrated *in vacuo*. **10** (48 mg, 38 %) was purified with ethyl acetate/hexane (1/50). ¹H NMR (CDCl₃): δ 1.52 (s, 6H), 3.54 (s, 3H), 5.25 (s, 2H), 5.71 (d, J = 9.67 Hz, 1H), 6.38 (d, J = 9.96 Hz, 1H), 7.26 (d, J = 1.76 Hz, 1H), 7.52 (d, J = 1.76 Hz, 1H), 9.80 (s, 1H).

***N*-(8-methoxymethoxy-2,2-dimethyl-2*H*-chromen-6-yl)methyl]-2-**

methylpropan-1-amine (11)—2-Methylpropan-1-amine (0.5 mL, 5 mmol) and *p*-toluenesulfonic acid monohydrate (110 mg, 0.6 mmol) were added to a solution of **10** (143 mg, 0.6 mmol) in 5 mL of methylene chloride. The reaction mixture was refluxed overnight and concentrated *in vacuo*. Disappearance of the aldehyde peak at 9.80 ppm (s, 1H) and appearance of the imine peak at 8.07 ppm (s, 1H) were confirmed by NMR.

The crude product was dissolved in 3 mL of a mixture of methylene chloride and toluene (1/2 (v/v)), to which 1 M DIBAL in toluene (3 mL, 3 mmol) was added slowly to generate gentle bubbling. The reaction mixture was stirred for 5 hours and 20 mL of 1 N aqueous HCl was added. The aqueous phase was extracted by methylene chloride (40 mL × 3). The combined organic layer was washed by brine, dried with magnesium sulfate, and then concentrated *in vacuo*.

11 (22 mg, 13 %) was purified by silica gel chromatography with triethylamine/methanol/methylene chloride (1/3/100). ¹H NMR (CDCl₃): δ 0.91 (d, *J* = 3.13 Hz, 6H), 1.46 (s, 6H), 1.75~1.82 (m, 1H), 2.43 (d, *J* = 7.04 Hz, 2H), 3.53 (s, 3H), 3.66 (s, 2H), 5.20 (s, 2H), 5.62 (d, *J* = 9.78 Hz, 1H), 6.30 (d, *J* = 9.78 Hz, 1H), 6.69 (d, *J* = 1.96 Hz, 1H), 6.96 (d, *J* = 1.96 Hz, 1H).

***N*-[8-hydroxy-2,2-Dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(propan-2-yl)-pyridine-2-sulfonamide (6h)—11** (30 mg, 0.1 mmol), pyridine-2-sulfonyl chloride (57 mg, 0.3 mmol), and *N,N*-diisopropylethylamine (0.2 mL, 1 mmol) were dissolved in 1 mL of methylene chloride, and stirred at room temperature overnight. A completion of the reaction was confirmed by disappearance of the amine spot by TLC with triethylamine/methanol/methylene chloride (1/3/100) and appearance of the product spot by TLC with ethyl acetate/hexane (1/1). ***N*-[8-methoxymethoxy-2,2-Dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(propan-2-yl)-pyridine-2-sulfonamide** (31 mg, 70 %) was purified by silica gel chromatography with ethyl acetate/hexane (1/1). ¹H NMR (CDCl₃): δ 0.78 (d, *J* = 6.45 Hz, 6H), 1.45 (s, 6H), 1.72~1.76 (m, 1H), 3.11 (d, *J* = 7.62 Hz, 2H), 3.50 (s, 3H), 4.40 (s, 2H), 5.14 (s, 2H), 5.62 (d, *J* = 9.96 Hz, 1H), 6.23 (d, *J* = 9.67 Hz, 1H), 6.60 (d, *J* = 1.76 Hz, 1H), 6.87 (d, *J* = 1.76 Hz, 1H), 7.43~7.47 (m, 1H), 7.84~7.96 (m, 2H), 8.68~8.7 (m, 1H).

***N*-[8-methoxymethoxy-2,2-Dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(propan-2-yl)-pyridine-2-sulfonamide** (31 mg, 0.07 mmol) was dissolved in a co-solvent of tetrahydrofuran (1 mL) and 6 N aqueous HCl (1 mL), and then stirred for 3 hours. 10 mL of water was added to the reaction mixture, and then tetrahydrofuran was evaporated. The remaining aqueous phase was extracted by methylene chloride (10 mL × 3), washed with brine, dried with anhydrous magnesium sulfate, and concentrated *in vacuo*. **6h** (19 mg, 49 %) was purified by silica gel chromatography with ethyl acetate/hexane (1/1). ¹H NMR (CDCl₃): δ 0.77 (d, *J* = 6.45 Hz, 6H), 1.44 (s, 6H), 1.72~1.79 (m, 1H), 3.11 (d, *J* = 7.62 Hz, 2H), 4.39 (s, 2H), 5.61 (d, *J* = 9.67 Hz, 1H), 6.25 (d, *J* = 9.96 Hz, 1H), 6.50 (d, *J* = 1.76 Hz, 1H), 6.65 (d, *J* = 1.76 Hz, 1H), 7.43~7.47 (m, 1H), 7.84~7.97 (m, 2H), 8.69 (d, *J* = 4.40 Hz, 1H).

HRMS *m/z* [M+Na]⁺ calcd 425.15055, found 425.15091.

HPLC-1: Flow rate: 2 mL/minute: *t_R* = 2.9 minute, Purity = 100%; Flow rate 1 mL/minute: *t_R* = 5.6 minute, Purity = 100 %.

HPLC-2: *t_R* = 5.172 minute, Purity = 100 %.

***N*-[8-hydroxy-2,2-Dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(propan-2-yl)-pyridine-4-sulfonamide (6l)—6l** was synthesized by the same method as that of **6h**.

Yield in two steps = 48 %.

¹H NMR (CDCl₃): δ 0.80 (d, *J* = 6.74 Hz, 6H), 1.45 (s, 6H), 1.75~1.82 (m, 1H), 2.98 (d, *J* = 7.62 Hz, 2H), 4.24 (s, 2H), 5.62 (d, *J* = 9.67 Hz, 1H), 6.22 (d, *J* = 9.67 Hz, 1H), 6.39 (s, 1H), 6.60 (s, 1H), 7.62 (d, *J* = 5.86 Hz, 1H), 8.81 (d, *J* = 4.98 Hz, 1H).

HRMS *m/z* [M+Na]⁺ calcd 402.16133, found 403.16924.

HPLC-1: Flow rate: 2 mL/minute: *t_R* = 2.7 minute, Purity = 98%; Flow rate: 1 mL/minute: *t_R* = 5.3 minute, Purity = 98 %

HPLC-2: *t_R* = 5.532 minute, Purity = 100 %.

4.2. Physicochemical property measurements

HPLC was measured with a Waters 1525 Binary HPLC pump coupled by Waters 2487 Dual λ Absorbance Detector and the data was processed by Waters Breeze GPC Software. 254 and 280 nm were used for UV absorbance measurements. Symmetry C₁₈, WAT045905 (5 μ m, 4.6 $\times$ 150 mm) column was used with an eluent (70: 30: 0.1 = methanol: water: triethylamine) with a flow rate, 2 mL/min. Standard curves of **1**, **6a**, **6d** and **6g** were constructed by injection of 2, 4, 6, 8, and 10 μ L of standard solutions in 100 % of methanol (0.01 g/mL) into the HPLC system. 1-Octanol (99 %) was purchased from Alfa Aesar and phosphate buffer (0.2 M, pH 7.4) was purchased from Electron Microscopy Sciences.

4.2.1. Log $P_{7.4}$ measurements⁶⁶—Log $P_{7.4}$ values of **6a** and **6g** were measured according to the “OECD GUIDELINE FOR TESTING OF CHEMICALS 107 adopted by the Council on 27th July 1995” (Method 1), and they were used as two reference materials for the rest of the compounds of which log $P_{7.4}$ values were measured by “OECD GUIDELINE FOR TESTING OF CHEMICALS 117 adopted by the Council on 13th April 2004” (Method 2). The first method was more direct but too complicated to be applied for all compounds, so it was used only for **6a** and **6g**, which were then used as reference materials with Aniline, benzene, toluene, chlorobenzene, in method 2 to increase its reliability.

Method 1: 70 mL of 1-octanol was stirred vigorously with 30 mL of phosphate buffer at room temperature for 24 hours, and the aqueous layer was discarded. The 1-Octanol layer was washed with 30 mL of phosphate buffer. The same procedure was applied to 70 mL of phosphate buffer and 30 mL of 1-octanol. 1 - 2 mg of **6a** (or **6g**) was dissolved in 2 mL of 1-octanol saturated with phosphate buffer in 20 mL glass tubes in duplicate. 2 mL of phosphate buffer saturated with 1-octanol was added to each tube, which were then tightly sealed with a cover. The mixtures were mechanically shaken for 15 minutes. The two layers were separated by centrifugation at 1,500 g for 10 minutes, and 1 mL of each layer was transferred to a glass vial. 0.1 mL of the 1-octanol layer was mixed with 5 mL of water and 10 mL of HPLC eluent (70: 30: 0.1 = methanol: water: triethylamine) to eliminate the 1-octanol effect on a retention time of HPLC, of which 0.5 mL was injected into the HPLC system. 0.5 mL of the aqueous layer was injected into the HPLC system. Peak areas at 254 nm were quantified by standard curves described above, and used for the log $P_{7.4}$ calculations.

Method 2: All thirteen compounds (**1**, **6a** ~ **6l**), aniline, benzene, toluene, and chlorobenzene were injected into the HPLC system described above. Retention times for a solvent and each compound were obtained. Aniline, benzene, toluene, chlorobenzene, **6a**, and **6g** were used as reference compounds to obtain coefficients of a linear regression. Log $P_{7.4}$ of all other compounds were obtained by the linear regression equation constructed from the reference compounds.

4.2.2. Aqueous solubility measurements—**1** (1 - 2 mg) was placed in a 10 mL glass tube, to which 2 mL of distilled water was added. The suspension was sonicated for 30 minutes at room temperature, and then centrifuged at 1,500 g at 20 °C for 5 minutes. The liquid was transferred to a clean glass tube, and then filtered through a polytetrafluoroethylene (PTFE) filter (pore size: 0.2 μ m) slowly. 500 μ L or 1 mL of the filtered clear solution was injected into the HPLC system, and peak areas at 254 nm were quantified using the standard curves described above. Three independent experiments were performed and the average value was calculated. The same procedure was used for **6a**, **6d**, and **6g**.

The aqueous solubilities of **1**, **6a**, and **6d**, were also independently measured by laser nephelometry. The compounds and an internal control were dissolved in 100 % of DMSO to obtain a final concentration of 30 mg/mL to make stock solutions. A stock solution was serially diluted (concentration profile: 30, 20, 15, 10, 7.5, 5, 2.5, 1.25, 0.63, 0.31, and 0.15 mg/mL) in test tubes with 100 % of DMSO. The concentration profile was transferred to 96 well microplates (Costar black clear bottom) and serially diluted to a final concentration of 1 % of DMSO and a final drug concentration of 300, 200, 150, 100, 75, 50, 25, 12, 6, 3 and 1.5 μ g/mL with phosphate buffered saline (pH 7.4, 5.0, or 3.0). The microplates were incubated for 90 minutes at ambient temperature. Laser nephelometry (NEPHELOstar, BMG Lab Technologies) was used to determine the point at which the solute began to precipitate out of solution.

4.3. Metabolism

1, **6a**, **6d**, **6g**, and **6h** (20 μ M) were individually incubated with fresh mouse plasma or liver homogenate in phosphate-buffered saline (pH 7.4) (1/2 (w/v)) at 37 °C with constant shaking. At predetermined time points, 50 μ L of plasma or liver homogenate sample was collected, and extracted with 100 μ L of acetonitrile. The supernatant was reduced to dryness under vacuum overnight and reconstituted in the HPLC mobile phase. The concentrations of each compound were individually determined using reverse phase HPLC methodology. The HPLC system (Waters, Milford, MA) consisted of a Waters 2795 pump, a Phenomenex (Torrance, CA) C₈ column (5 μ m, 4.6 $\times$ 150 mm), and a Waters 996 photodiode array detector. As an internal standard, α -naphthoflavone was used at a concentration of 2 μ M. The detection wavelengths for these compounds were: **1** at 250 nm, both **6a** and **6d** at 264 nm, both **6g** and **6h** at 268 nm, and α -naphthoflavone at 281 nm. The linear range for the standard curves of all compounds was between 1 – 50 μ M. All studies were done in triplicates.

4.4. HIF-dependent Luciferase Reporter Assays

The LN229-V6R and LN229-Lux cells were maintained in Dulbecco's Modified Eagle's Medium, DMEM (Mediatech, Herdon, VA) with 10% serum and antibiotics as previously described.⁴⁶ The cells (3×10^4 in 300 μ L of DMEM medium), were seeded in each well of 48-well plates, and cultured under normoxia (21 % O₂, 5 % CO₂, and 74 % N₂) for 24 hours. Subsequently, the medium of each well was replaced with 300 μ L of DMEM medium with 1 % of DMSO, without (control) or with heteroarylsulfonamides, **6a**, **6g**, and **6l** (at 2.5, 5.0, and 10.0 μ M), the plates further incubated at 37 °C for one hour, and then transferred to a Steri-cycle CO₂ incubator (Thermo Forma, Model 370) under hypoxia (1 % O₂, 5 % CO₂, and 74 % N₂) at 37 °C for another 24 hours. The cells were washed with PBS buffer, and a cell extract prepared after lysing them with 40 μ L/well of lysis buffer (Promega, part# E1944) for 10 minutes. Protein suspensions (20 μ L) from each well were mixed with 25 μ L of luciferase assay substrate (Promega, Part# E151A) and luminescence was measured using a 20/20n Luminometer (Turner BioSystems Inc.).² Assays were performed in triplicates.

4.5. Detection of cellular HIF-1 α protein levels by western blot analyses

Western blots were prepared as previously described.⁴⁶ Briefly, LN229-V6R cells (2×10^5 in 2 mL of DMEM) were seeded in 35 mm cell culture dishes, cultured under normoxia for 24 hours, and then new DMEM medium added with 1 % of DMSO, and heteroarylsulfonamides **6a**, **6g**, **6l** (at 3, 6, 12, 25, 50 or 100 μ M). Dishes were incubated under normoxia at 37 °C for one hour, and then transferred to hypoxia (1 % O₂) for 24 hours.

Cells were lysed with 1 $\times$ loading buffer (62.5 mM Tris-HCl (pH 6.8 at 25 °C), 2 % (w/v) SDS, 50 μ M 2-mercaptoethanol, 10 % glycerol, 0.01 % bromophenol blue), and proteins

were separated by electrophoresis in a 4 ~ 12.5 % Tris-HCl gradient gel (Bio-Rad Richmond, CA). The proteins were transferred onto a nitrocellulose membrane and immunoblotted using anti-HIF-1 α antibody (1: 500 dilution BD biosciences, San Diego, CA) and anti- β -actin antibody (1: 3,000 dilution, Santa Cruz Biotechnologies, Santa Cruz, CA). The secondary horseradish peroxide-conjugated antibodies were added and horseradish peroxide activities were measured by enhanced chemiluminescence (Pierce, Rockford, IL).

Western blotting of each heteroarylsulfonamide (**6a**, **6g** or **6l**) was repeated at least two times.

4.6. Measurement of cell growth by Sulforhodamine B (SRB) colorimetric assays^{68,69}

LN229-V6R cells (2×10^3 in 200 μ L DMEM) were plated in each well of 48-well plates. Cells were allowed to attach under normoxia for 24 hours, then the medium replaced with DMEM with 1 % of DMSO containing **6a**, **6g**, or **6l** at a concentration of 3, 6, 12, 25, 50, or 100 μ M. The plates were incubated under normoxia at 37 °C for one hour, and then under normoxia or hypoxia for 3 days. The individual assays were performed in quadruplicates or sextuplicates and the experiments repeated two independent times.

Cells were fixed by gentle addition of 50 μ L of cold (4 °C) 10 % of trichloroacetic acid (TCA), and then incubated at 4 °C for one hour. Each well was washed with deionized water three times, and then air-dried. Cells were stained for 30 minutes by addition of 100 μ L of SRB solution (0.04 % of SRB (w/v) in 1 % of acetic acid (v/v)) to each well. Each well was washed five times with 1 % of acetic acid, and air-dried for 30 minutes. Bound dye was solubilized with 10 mM of Tris base (pH 10) prior to measuring the Optical Density at 564 nm in a BioTek Synergy HT.

4.7. Clonogenicity Assays

Heteroarylsulfonamides (**6a**, **6g**, **6l**) were dissolved in DMSO at 10 mM concentration and serially diluted in DMEM medium to reach final concentrations of 6.25, 12.5, 25, 50 and 100 μ M with 1 % of DMSO. Human glioma cells (LN229), or immortalized human fibroblasts (HFF-1), were seeded at 200 or 1,000 cells per well in 6-well plates, and left to adhere on the plates under normoxia for 16 hours. The media were removed and DMEM media without (control) or with the compounds was added to each well. The plates were incubated under normoxia for one hour, and then under normoxia (21 % O₂) or hypoxia (1 % O₂) for 14 days. Thereafter, the cells were fixed and the number of colonies in each well revealed by staining with crystal violet (0.9 %).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the National Institute of Health grants P50 CA128301-01A1, Emory Molecular and Translational Imaging Research Center (EMTIC)/*In vivo* Cellular and Molecular Imaging Centers (ICMIC) (to MM), P50 CA128301-01A1 Pilot Project #2 (to JM), R01 CA116804 (to EGVM) and V Foundation, Max Cure and Samuel Waxman Cancer Research foundation grants (to EGVM).

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Abbreviations list

HIF-1	hypoxia inducible factor-1
HRE	hypoxia response element
LN229-V6R	LN229 cells transfected with a vector, pBI-GL HRE V6R that contains a HRE-driven promoter and a luciferase reporter gene
DMSO	dimethyl sulfoxide
NMP	<i>N</i> -methyl-2-pyrrolidone

(A) 3,4-Dimethoxy-*N*-[(2,2-dimethyl-2*H*-chromene-6-yl)methyl]-*N*-phenylbenzenesulfonamide (**1**)

(B) *N*-[(2,2-dimethyl-2*H*-chromene-6-yl)methyl]-*N*-(propan-2-yl)arylsulfonamide (**2**, **3**, **4**, **5**)

(C) *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]heteroarylsulfonamides

Figure 1.
Design of *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]heteroarylsulfonamides.

Figure 2.

Reporter assays measuring luciferase activity in extracts from cells containing hypoxia-inducible (A) or constitutive (B) luciferase reporter genes following treatment with the indicated heteroarylsulfonamides. Results are plotted as percent of luciferase activity found in extracts of untreated cells used as controls. (A) Luciferase activity in extracts of LN229-V6R cells, which contain a HIF-inducible luciferase reporter gene. The cells were grown under hypoxia to activate HIF. (B) Luciferase activity in extracts of LN229-Lux cells, which contain a constitutively expressed luciferase reporter gene. Results shown are from cells grown under normoxia; similar results were obtained under hypoxia.

Figure 3. Measurement of HIF-1α levels in LN229-V6R cells in response to various doses of **6a**, **6g**, and **6l** by western blot analysis. ^a controls contain vehicle only (1% DMSO)

Scheme 1.

Synthesis of *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]

heteroarylsulfonamides.^{a a} Reagents and conditions: a. 3-chloro-3-methyl-1-butyne, 4 N aq. NaOH, DMF, 60 °C, overnight; b. *N*-methylpyrrolidone (NMP), reflux, overnight (15 % in two steps); c. alkylamine (propan-2-amine, cyclopropanamine, 2-methylpropan-1-amine, 1-cyclopropylmethanamine, cyclobuthanamine, cyclopenthanamine), *p*-toluenesulfonic acid monohydrate, methylene chloride, reflux, overnight; d. diisobutylaluminum hydride (DIBAL), toluene, overnight (39 - 89 % in two steps); e. heteroarylsulfonyl chloride, triethylamine, methylene chloride, reflux, overnight, or heteroarylsulfonyl chloride, *N,N*-diisopropylethylamine, methylene chloride, 0 °C to room temperature, overnight (17 -77 %).

Scheme 2.

Synthesis of *N*-[(8-hydroxy-2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(2-methylpropan-1-yl)pyridine-2-sulfonamide (**6h**), and *N*-[(8-hydroxy-2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(2-methylpropan-1-yl)pyridine-4-sulfonamide (**6l**).^{a, a} Reagents and conditions: a. 3-methylbut-2-enal, pyridinium trifluoromethanesulfonate, pyridine, reflux, 2 days (2.4 %); b. chloromethyl methyl ether, *N,N*-diisopropylethylamine, tetrahydrofuran, reflux, overnight (38 %); c. 2-methylpropan-1-amine, *p*-toluenesulfonic acid monohydrate, methylene chloride, reflux, overnight; d. 1 M diisobutylaluminium hydride (DIBAL) in toluene, methylene chloride/toluene (1/2 (v/v)), overnight (13 % in two steps); e. pyridine-2-sulfonyl chloride, triethylamine, methylene chloride, reflux, overnight (for **6h**), or pyridine-4-sulfonyl chloride, *N,N*-diisopropylethylamine, methylene chloride, 0 °C to room temperature, overnight (for **6l**); f. 6 N aqueous HCl, tetrahydrofuran, 3 hours. (48 - 49 % in two steps).

Table 1

Molecular weights (M_w), $\log P$ s, and $\log S_w$ s of 3,4-dimethoxy-*N*-[(2,2-dimethyl-2*H*-chromene-6-yl)methyl]-*N*-phenylbenzenesulfonamide (**1**)⁴⁶, *N*-[(2,2-dimethyl-2*H*-chromen-6-yl)methyl]-*N*-(propan-2-yl)arylsulfonamides (**2**, **3**, **4**, **5**)⁵⁴, and twelve *N*-alkyl-*N*-[(8-*R*-2,2-dimethyl-2*H*-chromen-6-yl)methyl]heteroarylsulfonamides by *in silico* calculations. The values were calculated by on-line software, ALOGPS 2.1, Virtual Computational Chemistry Laboratory (<http://www.vcclab.org>).⁵⁷⁻⁶²

Compound Name	Chemical Structure	M_w (Molecular Weight)	$\log P$ (Lipophilicity)	$\log S_w$ (Aqueous Solubility)
1		465.61	4.94	-6.05

Compound Name	Chemical Structure	M_w (Molecular Weight)	$\log P$ (Lipophilicity)	$\log S_w$ (Aqueous Solubility)
2		371.54	4.37	-4.78
3		389.53	4.56	-4.79

Compound Name	Chemical Structure	M_w (Molecular Weight)	$\log P$ (Lipophilicity)	$\log S_w$ (Aqueous Solubility)
4		416.54	4.31	-5.07
5		431.60	4.13	-4.81

Compound Name	Chemical Structure	M_w (Molecular Weight)	$\log P$ (Lipophilicity)	$\log S_w$ (Aqueous Solubility)
6a		372.53	3.64	-3.73
6b		370.51	3.42	-3.89

Compound Name	Chemical Structure	M_w (Molecular Weight)	$\log P$ (Lipophilicity)	$\log S_w$ (Aqueous Solubility)
6c		386.56	4.03	-3.91
6d		384.54	3.75	-4.11
6e		384.54	3.77	-3.94

Compound Name	Chemical Structure	M_w (Molecular Weight)	$\log P$ (Lipophilicity)	$\log S_w$ (Aqueous Solubility)
6f		398.57	4.18	-4.20
6g		402.56	3.54	-3.74

Compound Name	Chemical Structure	M_w (Molecular Weight)	$\log P$ (Lipophilicity)	$\log S_w$ (Aqueous Solubility)
6h		402.56	3.59	-3.45
6i		386.56	3.78	-3.95
6j		389.57	3.45	-3.16

Compound Name	Chemical Structure	M_w (Molecular Weight)	$\log P$ (Lipophilicity)	$\log S_w$ (Aqueous Solubility)
6k		392.59	4.08	-4.19
6l		402.56	3.37	-3.39

$\log P_{7.4}$ aqueous solubility and hepatic metabolic stability of *N*-alkyl-*N*[(8-*R*-2,2-dimethyl-2-*H*-chromen-6-yl)methyl]heteroarylsulfonamides.

Table 2

Compound	$\log P_{7.4}$	Aqueous Solubility (n=3) ($\mu\text{g/mL}$, μM)	Half-life ^b (hour)	Remaining HIF-1 activity at 2.5 μM	
				% of control	Rank
1	3.7	0.003 ^d $\pm$ 0.001, 0.009 $\pm$ 0.003	11	28	1
6a	2.0	0.3 ^d $\pm$ 0.1, 1 $\pm$ 0.1	20	55	5
6b	3.0	nd ^c	nd	71	10
6c	4.1	nd	nd	85	13
6d	3.1	0.1 ^e $\pm$ 0.02, 0.2 $\pm$ 0.07	13	49	3
6e	3.0	nd	nd	53	4
6f	3.8	nd	nd	60	7
6g	1.3	22 $\pm$ 16, 80 $\pm$ 36	15	61	8
6h	1.3	nd	15	43	2
6i	3.6	nd	nd	63	9
6j	3.1	nd	nd	74	11
6k	4.4	nd	nd	78	12
6l	1.2	nd	nd	59	6

^a Aqueous solubility by laser nephelometry was measured as follows:

1: pH 7.4: < 15 $\mu\text{g/mL}$, pH 5.0: < 15 $\mu\text{g/mL}$, pH 3.0: < 15 $\mu\text{g/mL}$;

6a: pH 7.4: < 15 $\mu\text{g/mL}$, pH 5.0: 16 $\mu\text{g/mL}$, pH 3.0: 17 $\mu\text{g/mL}$;

6d: pH 7.4: < 15 μg .

^b Half-life was measured in homogenized mouse liver in PBS (1:2 (w/v)).

^c The notation "nd" signifies that an experiment was "not done".

Table 3

IC₅₀ (μM) values of SRB and clonogenicity assays. IC₅₀ values were calculated by fitting the data to exponential or polinomial equations with R² 0.8. The graphs were presented in the supporting information (S5).

(A) SRB Assay			
Compound Name	Condition	IC ₅₀ (μM)	
6a	Normoxia	100	
	Hypoxia	126	
6g	Normoxia	73	
	Hypoxia	146	
6l	Normoxia	92	
	Hypoxia	113	
(B) Clonogenicity Assays			
Compound Name	Cell Line	Condition	IC ₅₀ (μM)
6a	LN229	Normoxia	82
		Hypoxia	134
	HFF-1	Normoxia	62
		Hypoxia	93
6g	LN229	Normoxia	69
		Hypoxia	>100
	HFF-1	Normoxia	55
		Hypoxia	>100
6l	LN229	Normoxia	23
		Hypoxia	30
	HFF-1	Normoxia	22
		Hypoxia	40