Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

Design and Synthesis of Novel Small-Molecule Inhibitors of the Hypoxia Inducible Factor Pathway

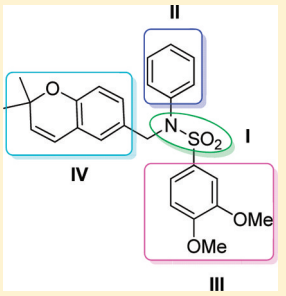
Suazette Reid Mooring,[†] Hui Jin,[†] Narra S. Devi,[‡] Adnan A. Jabbar,[§] Stefan Kaluz,[∥,‡] Yuan Liu,[∥] Erwin G. Van Meir,*[,‡,§,∥] and Binghe Wang*[,†]

[†]Department of Chemistry and Center for Diagnostics and Therapeutics, Georgia State University, Atlanta, Georgia 30302-4098, United States

[‡]Department of Neurosurgery and [§]Department of Hematology and Medical Oncology, Emory University School of Medicine, Atlanta, Georgia 30322, United States

[∥]Winship Cancer Institute, Emory University, Atlanta, Georgia 30322, United States

ABSTRACT: Hypoxia, a reduction in partial oxygen pressure, is a salient property of solid tumors. Hypoxia drives malignant progression and metastasis in tumors and participates in tumor resistance to radio- and chemotherapies. Hypoxia activates the hypoxia-inducible factor (HIF) family of transcription factors, which induce target genes that regulate adaptive biological processes such as anaerobic metabolism, cell motility, and angiogenesis. Clinical evidence has demonstrated that expression of HIF-1 is strongly associated with poor patient prognosis and activation of HIF-1 contributes to malignant behavior and therapeutic resistance. Consequently, HIF-1 has become an important therapeutic target for inhibition by small molecules. Herein, we describe the design and synthesis of small molecules that inhibit the HIF-1 signaling pathway. Many of these compounds exhibit inhibitory activity in the nanomolar range. Separate mechanistic studies indicate that these inhibitors do not alter HIF-1 levels but interfere with the ability of HIF-1α/HIF-1β to interact with cofactors p300/CBP to form an active transcriptional complex.



INTRODUCTION

Hypoxia is a hallmark of solid tumors, largely due to inadequate vascularization, and is characterized by a reduction in the partial oxygen pressure in cells or tissue.[1−3] Tumor hypoxia has been shown to reduce the effectiveness of radiation and chemotherapy.[4,5] The hypoxia inducible factor (HIF) family consists of the primary transcription factors activated by hypoxia and are responsible for orchestrating a number of cellular responses such as angiogenesis and glycolysis that help tumor cells adapt to hypoxic conditions.[6] HIFs are basic helix−loop−helix heterodimers composed of an oxygen sensitive HIF-α subunit and a constitutively expressed HIF-1β.[7] The levels of HIF-1α are determined by intracellular oxygen concentration. Under normoxic conditions, HIF-α is continually degraded by ubiquitination and proteosomal degradation. Degradation occurs when HIF-1α is hydroxylated at Pro 564 and Pro 402 located at its oxygen-dependent degradation domain (ODDD). This process is facilitated by a family of prolyl hydroxylases (PHDs) that require oxygen, iron, and 2-oxoglutarate as the cosubstrate to hydroxylate the specific amino acid residues.[8−10] After hydroxylation, HIF-1α binds to the von Hippel−Lindau protein (pVHL) which is part of an E3-ubiquitin ligase complex that marks HIF-1 for proteasomal degradation through ubiquitination.[11,12] Oxygen is required for the function of PHDs; therefore, under hypoxic conditions, HIF-1α is stabilized, accumulates, and translocates to the nucleus where it interacts with HIF-1β to form the active transcription factor HIF-1.[11,12] HIF-1 then specifically activates the transcription of over 100 selected genes by binding to hypoxia-responsive elements (HRE) on the gene regulatory DNA sequences.[13,14] The genes targeted for activation include those that encode enzymes that carry out anaerobic glycolysis, erythropoietin, a hormone that triggers red blood cell production, and vascular endothelial cell growth factor (VEGF), a powerful activator of new capillary formation and major driver of tumor angiogenesis.[15,16] Elevated levels of HIF-1α have been found in many human cancers,[17−19] and this is associated with poor response to treatment and patient mortality.[17,20−23] As a result, the HIF pathway has been exploited for the development of new cancer therapies,[24−27] including the development of small molecule inhibitors targeting HIF-1.[28−31]

To identify novel compounds for HIF-1 pathway inhibition, some 10 000 compounds from a 2,2-dimethylbenzopyran combinatorial library were screened.[32] The benzopyran moiety was chosen because it appears in more than 4000 natural products and is considered to be lipophilic enough to cross the blood−brain barrier.[33] The library was screened using a human glioma cell line containing an HRE-alkaline phosphatase reporter gene.[33,34] **1** (KCN-1) was identified as a potent inhibitor.[35] Further tests in animal models for cancer have shown its ability to inhibit cancer growth.[36,37] Separate mechanistic studies indicate that **1** does not alter HIF-1 levels but interferes with the ability of the HIF-1α/HIF-1β complex to associate with transcriptional cofactors p300/CBP (CREB-binding protein).[36] p300/CBP cofactors are transcription coactivating proteins. They

Received: July 30, 2011
Published: October 27, 2011

interact with various transcription factors such as HIF-1 and increase the expression of their target genes. Such a mechanism is different from HIF-1 inhibitors and may lead to significant insight into novel approaches to control solid tumor. With the desire to improve potency, our goal for this study was to build an SAR profile around the lead compound **1** in search of more potent and soluble small-molecule inhibitors of HIF-1-mediated transcription. This will help our effort in addressing the poor solubility of **1** and its need for cremophor/ethanol in formulation[36] which is associated with toxicity.[38]

RESULTS AND DISCUSSION

Design. The modification of compound **1** was approached in a systematic manner in which the molecule was divided into four regions as shown in Figure 1. In all, seven classes of compounds were designed and synthesized (Figure 2). The sulfonamide group of region I was either eliminated (class 1) or replaced by an amide group (class 7). Regions II and III were modified with several alkyl and aryl substitutions, using known synthetic procedures (class 2). The aldehyde derivative of the core structure underwent reductive amination with a variety of alkyl and aryl primary amines to provide modifications to region II. Then sulfonylation of the resulting secondary amines with various sulfonyl chlorides allowed modifications to region III. The benzopyran ring of region IV was probed to determine the influence of subtle and major modifications of this region on activity. The first modification was the replacement of the *gem*-dimethyl group of region IV with the 2-ethyl-2-methyl group (class 3). The benzopyran ring of region IV was also replaced with a quinoline ring (class 4) and benzofuran ring (class 5). Finally, the benzopyran ring of region IV was replaced with a pyranopyridine fused ring to give classes **6a**, **6b**, and **6c**.

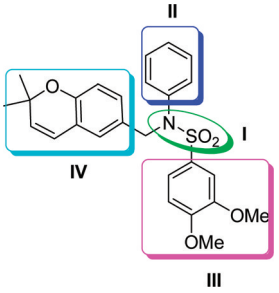


Figure 1. Four regions for modification of **1**. IC_{50} = 0.65 ± 0.09 μM (n = 42).

Chemistry. *Class 1: Benzopyran Analogues A.* For the synthesis of the class 1 analogues, the benzopyran moiety was retained while the sulfonyl and 3,4-dimethoxyphenyl groups were eliminated. To afford these analogues, the aldehyde derivative of the benzopyran moiety was synthesized followed by reductive amination and methylation of the resulting secondary amine (in some cases).

The synthesis of class 1 analogues began with 2,2-dimethyl-2*H*-chromene-6-carbaldehyde that was synthesized according to literature procedures.[39] Reductive amination of 2,2-dimethyl-2*H*-chromene-6-carbaldehyde with several primary amines gave analogues **2**. Methylation of secondary amine **2** with MeI and NaH generated analogues **3** (Scheme 1).

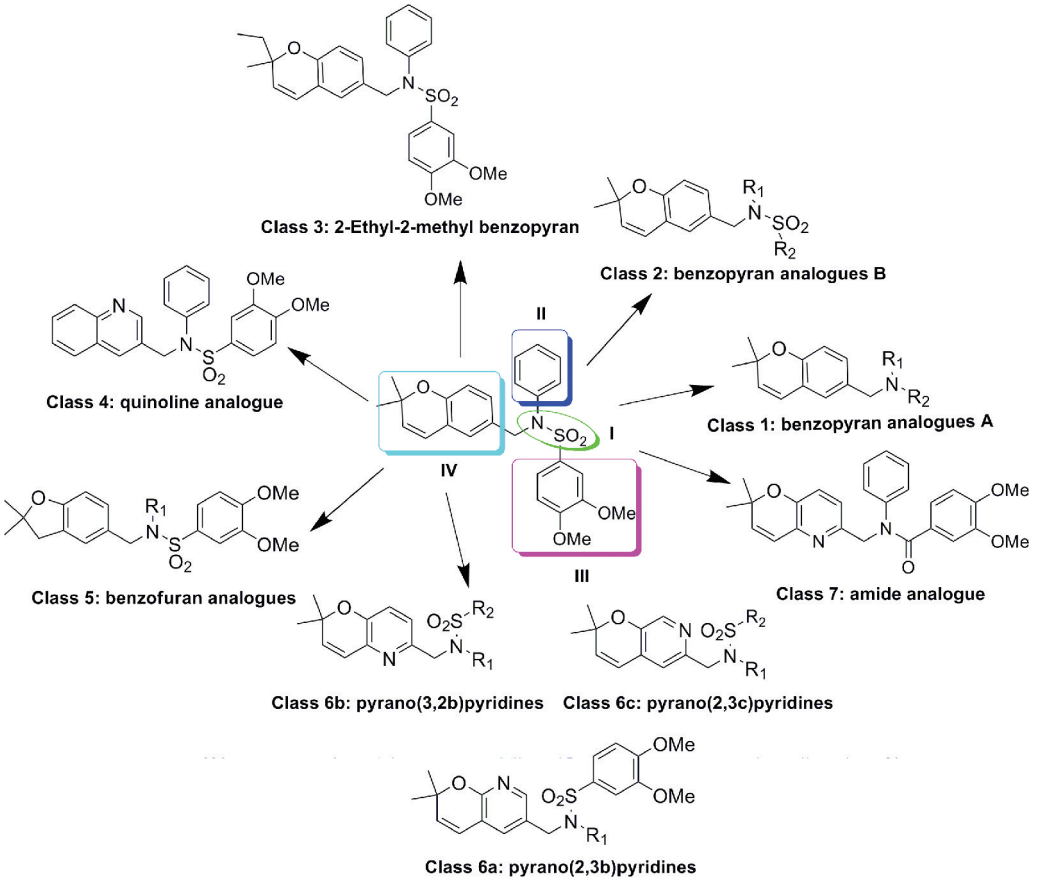
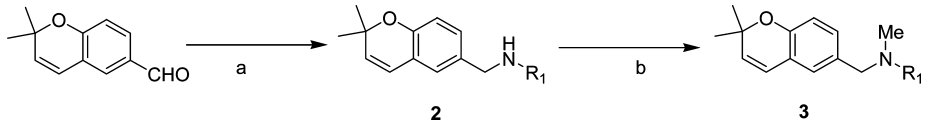


Figure 2. Analogues designed and synthesized.

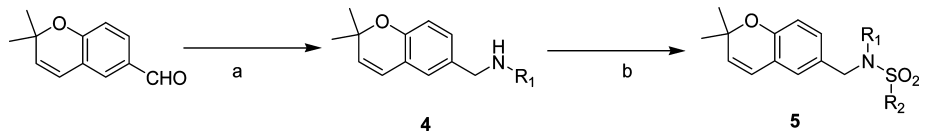
dx.doi.org/10.1021/jm201018g | *J. Med. Chem.* 2011, 54, 8471–8489

Scheme 1. Synthesis of Benzopyran Analogues A[a]



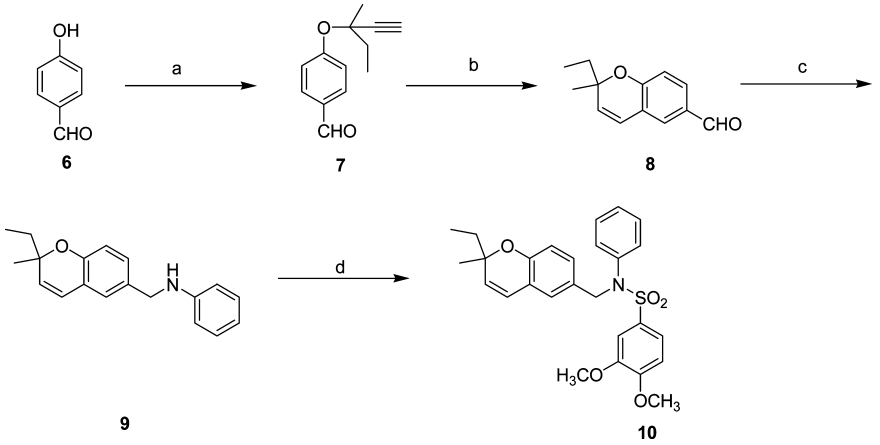
[a]R_1 = 3,4-dimethoxyphenyl (**2a**, **3a**), 2-pyridinyl (**2b**, **3b**), 2,4-dimethylphenyl (**2c**, **3c**), 4-carboxyphenyl (**2d**), 2-bromophenyl (**2e**), 2-fluorophenyl (**2f**). Reagents and conditions: (a) R_1NH_2, $ZnCl_2$, $NaCNBH_3$, room temp, 11−70%; (b) MeI, NaH, THF, room temp, 50−81%.

Scheme 2. Synthesis of Benzopyran Analogues B[a]



[a]R_2 = 3,4-dimethoxyphenyl and R_1 = phenyl (**1**), isopropyl (**5a**), propargyl (**5b**), butyl (**5c**), *t*-butyl (**5d**), allyl (**5e**), isobutyl (**5f**), cyclopentyl (**5g**), cyclopropyl (**5h**), cyclohexyl (**5i**). R_1 = phenyl and R_2 = 4-methoxyphenyl (**5j**), 3,5-dimethylphenyl (**5k**), 2,5-dichlorophenyl (**5l**), 2-trifluoromethoxy-4-bromophenyl (**5m**). Reagents and conditions: (a) R_1NH_2, $ZnCl_2$, $NaCNBH_3$, room temp, 60−70%; (b) R_2SO_2Cl, Et_3N, DCM, room temp, 30−95%.

Scheme 3. Synthesis of 2-Ethyl-2-Methylbenzopyran Analogue[a]



[a]Reagents and conditions: (a) 3-methyl-pent-1-yn-3-ol DBU, TFAA, CuCl, CH_3CN, 0 °C to room temp, 30%; (b) xylene, microwave (220 W, 200 Torr, 120 °C, 100 min); (c) aniline, $ZnCl_2$, $NaCNBH_3$, room temp, overnight, 41%; (d) 3,4-dimethoxybenzylsulfonyl chloride, Et_3N, DCM, room temp, 24 h, 32%.

Class 2: Benzopyran Analogues B. Next, modifications were separately made to region II (**5a**−**i**) and then to region III (**5j**−**m**) of compound **1** with various alkyl and aryl substituents in order to probe their effect on activity. Reductive amination of 2,2-dimethyl-2*H*-chromene-6-carbaldehyde with various aryl- or alkylamines afforded compound **4** that was subsequently converted to sulfonamides with various sulfonyl chlorides to give analogues **5** (Scheme 2).

Class 3: 2-Ethyl-2-methylbenzopyran Analogues. Next, class 3 analogues were generated that involved a slight modification to the benzopyran portion (region IV) of **1**. The ethyl group replaced one of the *gem*-dimethyl groups on the benzopyran ring (Scheme 3). For the synthesis of these analogues, O-alkylation of 4-hydroxybenzophenone **6** with 3-methylpentyn-3-ol afforded compound **7**. Claisen rearrangement and rearomatization of **7** by microwave irradiation yielded compound **8**. Reductive amination of aldehyde **8** gave the secondary amine **9** that was converted to the corresponding sulfonamide **10** with 3, 4-dimethoxybenzenesulfonyl chloride.

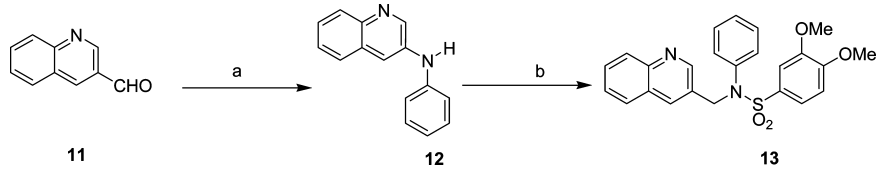
Class 4: Quinoline Analogues. The next modification to region IV was the replacement of the benzopyran ring with another fused ring system: quinoline (Scheme 4). Commercially available quinoline aldehyde **11** was subjected to reductive amination, followed by sulfonylation to afford compound **13** in 45% yield.

Class 5: Benzofuran Analogues. Additionally, the 2,2-dimethylbenzopyran ring (region IV) of **1** was replaced with a 2,2-dimethylbenzofuran ring (Scheme 5). Commercially available 2,2-dimethyl-2,3-dihydrobenzofuran-5-carbaldehyde **14** was subjected to reductive amination with various primary amines to give compound **15** and then sulfonylation with 3,4-dimethoxybenzenesulfonyl chloride to give analogues **16**.

Class 6: Pyranopyridines. We also replaced one of the carbons on the aromatic portion of the benzopyran ring with nitrogen to afford pyranopyridine analogues. The pyridine nitrogen was separately placed in each of the three available positions on the benzopyran ring. It was envisioned that these compounds would provide increased water solubility and additional interaction points and therefore increased activity.

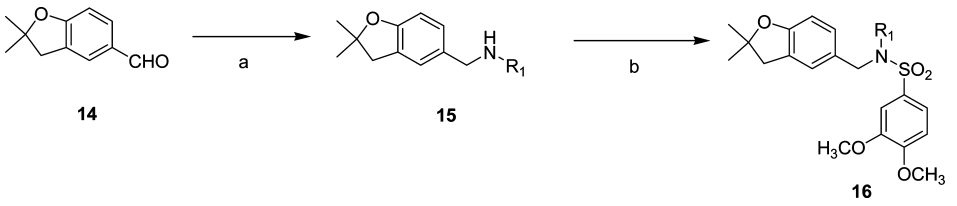
dx.doi.org/10.1021/jm201018g | *J. Med. Chem.* 2011, 54, 8471−8489

Scheme 4. Synthesis of Quinoline Analogue[a]



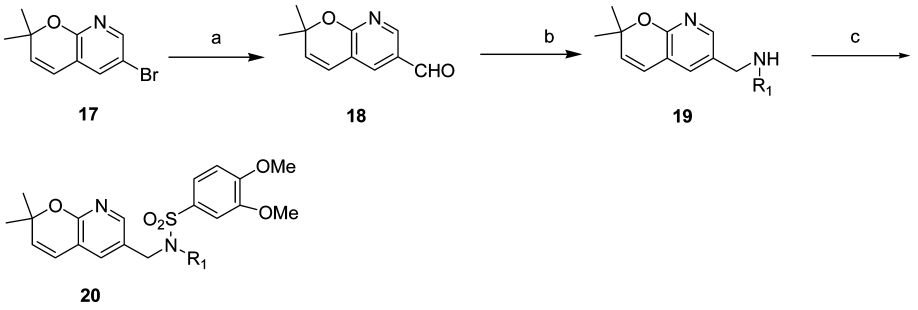
[a]Reagents and conditions: (a) aniline, $ZnCl_2$, $NaCNBH_3$, room temp, 64%; (b) 3,4-dimethoxybenzylsulfonyl chloride, pyridine, room temp, 45%.

Scheme 5. Synthesis of Benzofuran Analogues[a]



[a]R_1 = phenyl (**16a**), cycloheptyl (**16b**), isopropyl (**16c**), butyl (**16d**), cyclohexyl (**16e**), cyclopentyl (**16f**). Reagents and conditions: (a) R_1NH_2, $ZnCl_2$, $NaCNBH_3$, room temp, 2 h; (b) 3,4-dimethoxybenzenesulfonyl chloride, Et_3N, DCM, room temp, 14−42%.

Scheme 6. Synthesis of Pyrano[2,3-*b*]pyridine Analogues[a]



[a]R_1 = phenyl (**20a**), cyclohexyl (**20b**). Reagents and conditions: (a) (i) BuLi, −78 °C; (ii) DMF, anhydrous ether, 31%; (b) R_1NH_2, $ZnCl_2$, $NaCNBH_3$, MeOH, 49%; (c) 3,4-diethoxybenzenesulfonyl chloride, Et_3N, DCM, room temp, 43−60%.

The incorporation of a nitrogen atom may also decrease the electron density and make it more stable toward oxidative metabolism.

The first set of these compounds was the pyrano[2,3-*b*]pyridines **20**. The 2*H*-pyrano[2,3-*b*]pyridine core **17** was synthesized as previously described.[40] Formylation of **17** with BuLi and DMF gave compound **18**. Reductive amination with aniline (**19a**) or cyclohexylamine (**19b**) followed by sulfonylation with 3,4-dimethoxybenzesulfonyl chloride afforded compounds **20a** and **20b** (Scheme 6).

The second set of analogues in this class was the pyrano-[3,2-*b*]pyridines that were prepared using the following procedure: O-alkylation of commercially available 2-bromo-5-hydroxypyridine **22** followed by Claisen rearrangement and formylation gave compound **24** with a 23% overall yield for the two steps. Subsequent reductive amination of **24** and then reaction of secondary amine **25** with various sulfonyl chlorides afforded analogues **26** (Scheme 7).

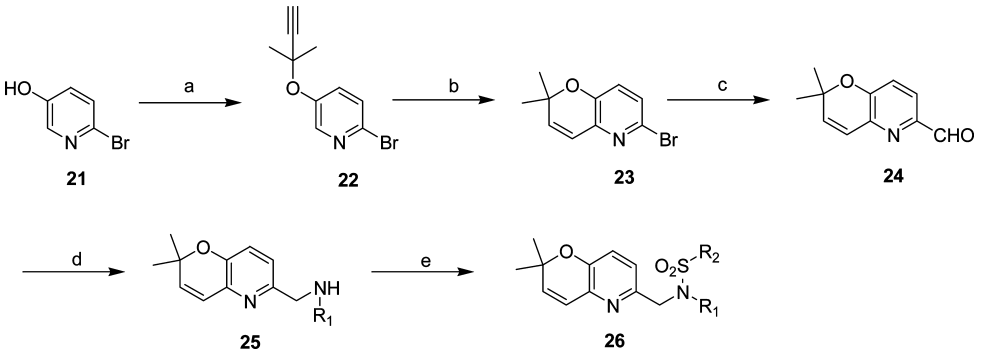
The final pyranopyridine derivative was the pyrano[2,3-*c*]-pyridines (class 6c). To synthesize these analogues, 2-hydroxy-5-methylpyridine **27** was brominated to afford compound **28**.[41] N-Oxidation of **28** with m-CPBA gave product **29** in 70% yield. Rearrangement of **29**, facilitated by TFAA, afforded compound **30**. O-Alkylation of **30** with 3-chloro-3-methyl-1-butene followed by Claisen rearrangement gave compound **32**. Nucelophilic substitution of the primary alcohol **32** with bromine gave compound **33**. Subsequent nucleophilic substitution of **33** with various primary amines followed by removal of the bromine with BuLi afforded compound **35**. Next sulfonylation of **35** with arylsulfonyl chlorides resulted in analogues **36** (Scheme 8).

Class 7: Amide Analogue. Finally, we replaced the sulfonamide of compound **26a** with an amide group. The amide group is a common bioisostere for sulfonamide and may enhance activity. In this case, the previously synthesized **25a** was reacted with 3,4-dimethoxybenzoyl chloride in the presence of triethylamine to give the product **37** with a 98% yield (Scheme 9).

Biology. The synthesized analogues of **1** were evaluated for their potential to inhibit HIF-1-mediated transcription under hypoxia (1% O_2) using a human glioma cell line LN229-HRE-Lux,[35] which stably expresses a hypoxia-responsive luciferase reporter gene (Tables 1−9). The IC_{50} values of all compounds were calculated based on a concentration curve testing of compounds at 0, 1, 5, 10, and 25 μM. The compounds were tested in single (n = 1) or multiple (n > 1) independent experiments each carried out in quadruplicate. Compound **1** was always tested along with the new analogues and has an IC_{50} of 0.65 ± 0.09 μM (n = 42)[35] using this cell-based reporter assay (Figure 1).

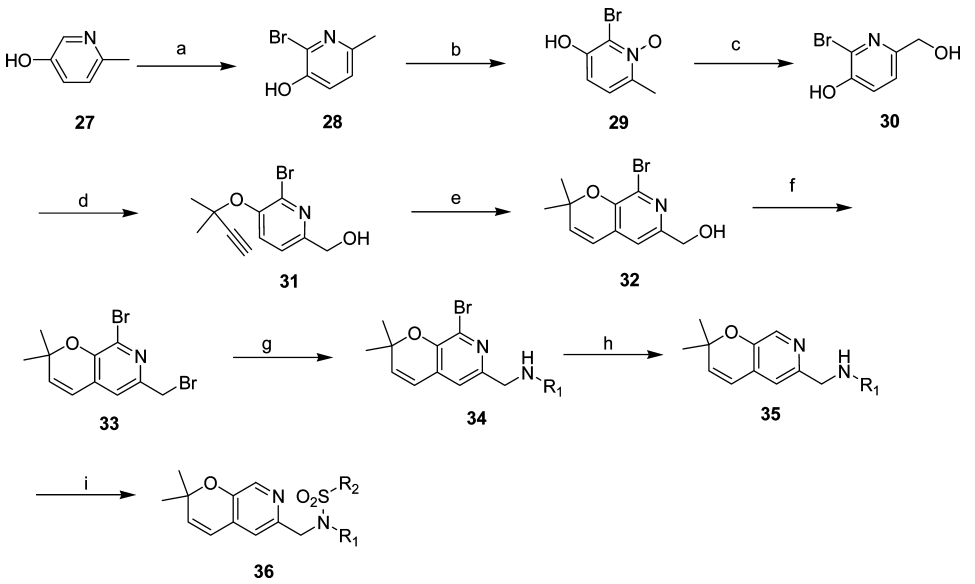
dx.doi.org/10.1021/jm201018g | J. Med. Chem. 2011, 54, 8471−8489

Scheme 7. Synthesis of Pyrano[3,2-*b*]pyridine Analogues[a]



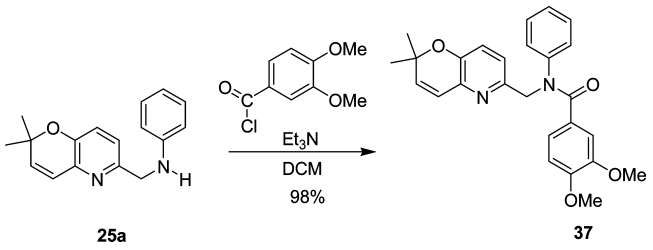
[a]R_2 = 3,4-dimethoxyphenyl and R_1 = phenyl (**26a**), butyl (**26b**), 3,4-dimethoxyphenyl (**26c**), cyclopentyl (**26d**), cyclohexyl (**26e**), tetrahydranapthyl (**26f**), cycloheptyl (**26g**), cyclooctyl (**26h**), cyclobutyl (**26i**), *p*-fluorophenyl (**26j**). R_1 = phenyl and R_2 = cyclohexyl (**26k**), isopropyl (**26l**), cyclopropyl (**26m**), butyl (**26n**), propyl (**26o**), isobutyl (**26p**), 4-biphenyl (**26q**), benzodioxolyl (**26r**), quinolin (**26s**), 2,3-dihydrobenzo(1,4)-dioxinyl (**26t**). Reagents and conditions: (a) 2-methylbut-3-yn-2-ol, TFAA, DBU, CH_3CN; (b) xylene, microwave heating, 120 °C, 30 min, 23% for two steps; (c) (1) BuLi, (2) DMF, anhydrous THF, 23%; (d) R_1NH_2, $ZnCl_2$, $NaCNBH_3$, MeOH; (d) R_2SO_2Cl, Et_3N, DCM, 40−65% for two steps.

Scheme 8. Synthesis of Pyrano[2,3-*c*]pyridine Analogues[a]



[a]R_1 = phenyl and R_2 = 4-methoxyphenyl (**36a**), 4-nitrophenyl (**36b**). R_1 = cyclohexyl and R_2 =4-isopropylphenyl (**36c**), 3,4-dimethoxyphenyl (**36d**). Reagents and conditions: (a) Br_2, pyridine, 0 °C, 74%; (b) *m*-CPBA, THF, 70%; (c) (1) TFAA, (2) MeOH, 30%; (d) 3-chloro-3-methyl-1-butene, K_2CO_3, KI, $CuCl_2$, acetone, 57%; (e) CuCl, toluene, microwave heating (200 W, 120 °C, 1 h), 70%; (f) CBr_4, PPh_3, DCM, 40%; (g) DIEA, DMF, 60 −78%; (h) BuLi, THF, −78 °C, 50−70%; (i) R_2SO_2Cl, pyridine, room temp, 70−89%.

Scheme 9. Synthesis of Compound 37

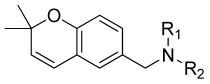


Class I (benzopyran A) analogues were designed to probe the importance of the sulfonyl group. In general, removal of the sulfonyl group in compounds **2a−f** and **3a−c** resulted in a marked decrease in activity (Table 1). For secondary amine compounds **2a−f**, only **2a** and **2b** had IC_{50} values below 10 μM; the others were higher than 25 μM. The best compound in that series was the 3,4-dimethoxyphenyl derivative **2a** with an IC_{50} of 3.0 μM. Analogues **3a−c** showed similar IC_{50} values as their secondary amine counterparts **2a−c**, with the exception of the 2,4-dimethoxyphenyl derivative **3c** that had an IC_{50} of 2.6 μM. Therefore, methylation of the secondary amine had no effect. As a result, it was concluded that the sulfonyl group was essential to the activity of these compounds and was retained in future modifications of compound **1**.

Next, region II of the molecule was probed with various alkyl and aryl substituents (**5a−k**). All the compounds were active to some extent (Table 2). The best of this group was the propargyl derivative **5b**, isobutyl derivative **5f**, and the cyclopropyl derivative **5h** with IC_{50} values of 1.3, 1.6, and 1.5 μM, respectively.

dx.doi.org/10.1021/jm201018g | *J. Med. Chem.* 2011, 54, 8471−8489

Table 1. Structures and Activities of Analogues 2a to 3c



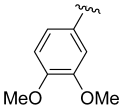
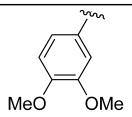
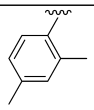
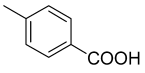
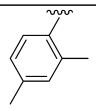
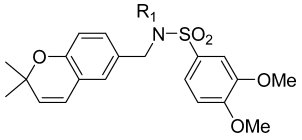
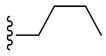
Compound	R_1	R_2	IC_{50} (μM)	Compound	R_1	R_2	IC_{50} (μM)
2a	H		3.0	**2f**	H		>25
2b	H		8.5	**3a**	Me		5.0
2c	H		>25.	**3b**	Me		8.4
2d	H		>25	**3c**	Me		2.6
2e	H		>25				

Table 2. Structures and Activities of Analogues 5a−i



Compound	R_1	IC_{50} (μM)	Compound	R_1	IC_{50} (μM)
5a		3.1	**5e**		3.4
5b		1.3	**5f**		1.6
5c		3.3	**5g**		0.5
5d		3.5	**5h**		1.5
			5i		4.0

In general, longer branched alkyl chains such as the isobutyl group of **5f** (1.6 μM) tended to do better than long unbranched chains such as the butyl group of **5c** (3.3 μM) or shorter branched chains such as the *tert*-butyl group of **5d** (3.5 μM). Also, alkyl rings smaller than six carbons were better tolerated.

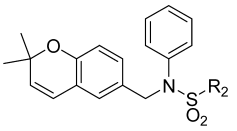
Compounds **5j−m** were modified at region III of **1** with various aryl substitutions (Table 3). The best compound in this group was the 4-methoxyphenyl substituted **5j** and 3,5-dimethylphenyl substituted **5k** with IC_{50} values of 0.6 and 0.5 μM, respectively. The 2-trifluoromethoxy-4-bromophenyl substitution (**5m**) resulted in a significant decrease in activity

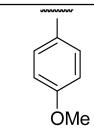
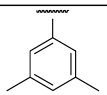
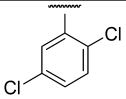
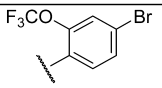
Compound **10** represented a subtle change to region IV of **1**. In this case, simply substituting one of the gem-dimethyls of the benzopyran ring system of **1** with an ethyl group resulted in a decrease in activity with an IC_{50} of 2.2 μM (Table 4). In the case of compound **13**, replacement of the benzopyran ring of **1** with a quinoline ring led to a reduction in HIF-1 inhibitory activity with an IC_{50} of 3.5 μM (Table 4).

The benzofuran derivatives **16** afforded some potent compounds (Table 5). A comparison of compound **16a** (IC_{50} = 0.5 μM) to **1** shows that the substitution of the benzopyran ring with benzofuran did not necessarily result in a much more potent compound than **1**, but the benzofuran analogue was comparable to that of **1**. The foreseeable benefit of the benzofuran structure of **16** is that it eliminates the double bond on the pyran ring of **1**. Since that double bond may be

dx.doi.org/10.1021/jm201018g | *J. Med. Chem.* 2011, 54, 8471−8489

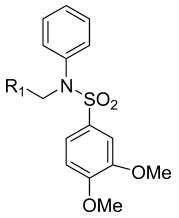
Table 3. Structures and Activities of Analogues 5j−m[a]

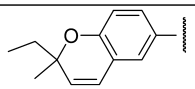
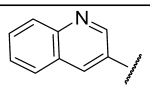


Compound	R_2	IC_{50} (μM)
5j		0.6
5k		0.5
5l		2.1
5m		>25

[a]Results were from single runs.

Table 4. Structures and Activities of Analogues 10 and 13[a]



Compound	R_1	IC_{50} (μM)
10		2.2
13		2.2

[a]Results were from single runs.

susceptible to epoxidation in vivo and thereby introduce toxicity, the benzofuran ring may be a better alternative. The ring size of the cycloalkyl derivatives seems to have an effect on activity. A comparison of the cycloheptyl ring of **16b** (9.1 μM), the cyclohexyl ring of **16e** (8.2 μM), and the cyclopentyl ring of **16f** (0.4 μM) seems to suggest that smaller rings (ring size 5 or smaller) tend to be more favorable than large rings (six carbons or more). This is similar to the trend seen with the benzopyran analogues B (class 2).

The first of the pyranopyridine analogues was class 6a, the pyrano[2,3-*b*]pyridines. Two compounds were synthesized in this class. Compound **20a** had the same substitutions as compound **1** with the exception of the pyranopyridine core. This compound showed modest activity with an IC_{50} of 2.5 μM. However, it was not as potent as **1**. Replacing the phenyl ring by the cyclohexyl ring (**20b**) resulted in a loss of activity; that is, the IC_{50} was higher than 25 μM (Table 6).

The next group of compounds in this class was the pyrano[3,2-*b*]pyridine (class 6b). Compounds **26a−j** were modified at region I with various alkyl- and arylamines. This class of compounds was among the best in the pyranopyridine class (Table 7). All of these compounds inhibited HIF-mediated transcription with the exception of the 2,4-dimethoxy derivative **26c**. Phenyl derivative **26a** is similar in structure to **1** with the exception of the pyrano[3,2-*b*]pyridine core. This compound had an IC_{50} of 1.3 μM, which is within the range of activity observed for **1**. Replacement of the phenyl group of **26a** with a cyclopentyl group (**26d**) and cyclohexyl group (**26e**) was effective. The best compound in this group (**26a−t**) was the cyclobutyl derivative **26i** with an IC_{50} of 0.25 μM. It is noted that **26i** was tested many times in independent experiments (n = 30) side by side with **1** and consistently showed ∼2.5-fold higher potency than **1**. When all the cylcoalkyl analogues were compared, the general trend remained about the same as that of other series, in that smaller rings (<6 carbons) tend to have better activity than larger ring derivatives (>6 carbons). The tetrahydronaphthalene derivatives **26f** had the lowest activity in this series with IC_{50} of 6.15.

Additionally, these pyrano[3,2-*b*]pyridines **26k−t** were also modified at region III with alkyl- and arylsulfonyl derivatives. Generally, this group did not produce very active compounds (Table 8). The cyclohexyl group was well tolerated in this position, leading to derivative **26k**, with an IC_{50} of 0.4 μM. In this case, the cyclopropyl derivative (**26m**) showed almost no activity within the experimental conditions. Compounds **26r** and **26t** were an attempt to fix the conformation of the 2,4-dimethoxybenzyl group to see if this modification would enhance activity. Compound **26r** that separated the oxygen atoms by one carbon showed a decrease in activity (IC_{50} = 6.5 μM) compared to the 3,4-dimethoxy substituted compound **26a** (1.3 μM). However, **26t**, in which two carbons separate the oxygen atoms, showed an IC_{50} of 0.9 μM. Also noted is the quinoline derivative **26s** that showed relatively good activity with an IC_{50} of 0.9 μM.

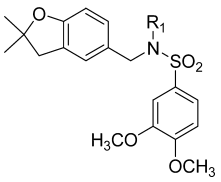
The third group of compounds in class 6 was the pyrano[2,3-*c*]-pyridinyl derivatives (class 6c). These compounds also showed good activities, which were comparable to **1** (Table 9). Derivatives **36a**, **36b** and **36d** all showed similar IC_{50} values of 1.4, 1.8 and 1.1 μM, respectively. The isopropylphenyl derivative **36c** showed a 5-fold decrease in activity when compared to the 3,4-dimethoxy derivative **36d**.

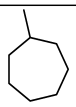
Since the pyranopyridine analogues were among the most potent compounds, we decided to replace the sulfonamide group with an amide group to see what effect this modification would have on activity. An amide is a commonly used bioisostere for sulfonamide. The amide derivative showed a 2-fold increase in activity over the sulfonamide derivative (Figure 3). This amide group can be incorporated in future modifications of the compound.

Following chemical optimization, the best compounds were subsequently retested in the luciferase reporter assay in a dose−response fashion to establish an IC_{50} of inhibitory activity on HIF transcription (Figure 4 and data not shown). Four-parameter logistic function was used to describe the dose−response relationship, and the data were fitted into the nonlinear mixed effects[42] model by nlme library in R.[43] Each compound was tested a minimum of 6 times on different days. To address variations from experiment to experiment, we used the nonlinear mixed effects model in which IC_{50} was set to have

dx.doi.org/10.1021/jm201018g | J. Med. Chem. 2011, 54, 8471−8489

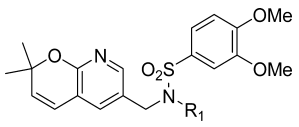
Table 5. Structures and Activities of Analogues 16a–f[a]

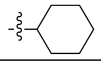


Compound	R_1	IC_{50}	Compound	R_1	IC_{50}
16a		0.5	**16d**		0.6
16b		9.1	**16e**		8.2
16c		1.5	**16f**		0.4

[a]Results were from single runs.

Table 6. Structures and Activities of Analogues 20a and 20b



Compound	R_1	IC50 (μM)
20a		2.5
20b		>25

an associated random effect. The results indicate that five compounds displayed IC_{50} values in the submicromolar range (Table 10), with **16a** and **26i** being the most promising, as they both showed more than 2-fold improvement over **1**.

We further confirmed inhibitory activity of these compounds on the HIF pathway by conducting additional experiments with the hypoxia-responsive promoter of the endogenous HIF transcriptional target gene vascular endothelial growth factor (*VEGF*). Using LN229 glioma cells stably transfected with a *VEGF* promoter-luciferase reporter (LN229-VEGF-Luc), we found that the tested compounds at 10 μM all significantly inhibited hypoxia-induced transcription from the *VEGF* promoter (Figure 5).

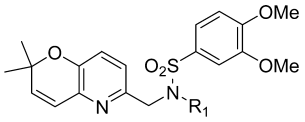
For further mechanistic studies, we picked the representative compounds and previously characterized HIF pathway inhibitors (**1**, **38** (Figure 6)[33] and bortezomib) as controls to evaluate their molecular basis of action using biochemical techniques. As HIF regulation typically occurs at the protein level, we probed by Western blotting whether the selected compounds had a direct effect on HIF-1α protein accumulation under hypoxia. HIF-1α levels were examined in cell extracts from cells grown under hypoxia in the presence or absence of inhibitor (20 μM). As expected, the results show that bortezomib, a proteasome inhibitor, leads to the accumulation of HIF-1α in an inactive form,[44] whereas **38**, a HIF-1α translation inhibitor, leads to a blockage of HIF-1α accumulation under hypoxia.[33] It was found that some of the compounds **5g** and **16a** reduced the level of expression of HIF-1α at 20 μM, whereas the remaining compounds did not (Figure 7). These data suggest that inhibition of HIF-1α expression is not a general cause of the strong inhibition seen against HIF-mediated transcription in the reporter assay. Such results suggest that at least for some of the compounds, **16d**, **16f**, and **26i**, the main biological activity is not mediated by inhibiting HIF-1α gene expression or affecting HIF-1α turnover through a blockage in translation of HIF-1α mRNA, or accelerated protein degradation. Instead, these findings imply that these inhibitors render the HIF transcriptional complex functionally inactive. Potential mechanisms may involve protein misfolding, incomplete protein modifications, and/or lack of HIF complex assembly. To dissect the precise mechanism of action of this class of HIF pathway inhibitors, additional work is needed. Ongoing mechanistic studies indicate that **1** does not alter HIF-1 levels but interferes with the ability of the HIF-1α/HIF-1β complex to associate with transcriptional cofactors p300/CBP,[36] and we anticipate that this will be similar for the new analogues identified here.

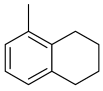
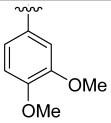
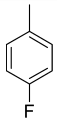
CONCLUSION

Several potent analogues of the lead compound **1** were synthesized. These analogues were able to yield information on the important functional groups at each of the four regions identified in Figure 1. General conclusions about the SAR of this molecule were determined (Figure 8). Analogues **2a–2f** demonstrated that the sulfonyl group was required for the activity of these compounds. Also, alkyl rings of five carbons or shorter, as well as longer branched chains, were well tolerated at region II of **1**. At region III, aryl substitutions seem to be better than alkyl substitutions. The benzofuran analogues (**16**) were also successful in that analogues in this series showed activity comparable to that of **1**. These benzofuran analogues may be a good future alternative to the benzopyran derivatives, since they do not contain electron-rich double bonds. To date, the pyrano[3,2-*b*]pyridine analogues (**26**) provided the most improvement in activity compared to **1**. The best overall

dx.doi.org/10.1021/jm201018g | J. Med. Chem. 2011, 54, 8471–8489

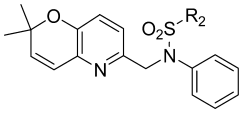
Table 7. Structures and Activities of Analogues 26a–j[a]

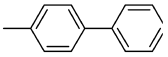
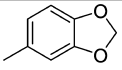
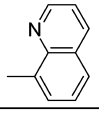
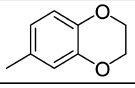


Compound	R_1	IC_{50} (μM)	Compound	R_1	IC_{50} (μM)
26a		1.3	**26f**		6.2
26b		0.9	**26g**		6.6
26c		>25	**26h**		0.7
26d		0.6	**26i**		0.28
26e		0.8	**26j**		5.7

[a]Results were from single runs.

Table 8. Structure and Activities of Analogues 26k–t[a]



Compound	R_2	IC_{50} (μM)	Compound	R_2	IC_{50} (μM)
26k		0.4	**26p**		0.9
26l		13.4	**26q**		3.4
26m		>25	**26r**		6.5
26n		5.0	**26s**		0.9
26o		6.4	**26t**		0.9

[a]Results were from single runs.

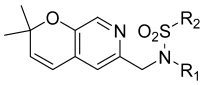
compound came from this group, the cyclobutyl derivative **26i**, which had an IC_{50} of 280 nM. The improvement in activity of these analogues over **1** may be a result of increased hydrophilicity and/or hydrogen bond interactions as a result of the addition of the pyridine ring. Overall, these small molecules show potential as effective HIF-1 inhibitors for antitumor therapy. These compounds can be especially useful in tumors that exhibit hypoxic resistance to chemotherapy and radiotherapy.

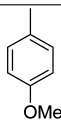
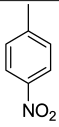
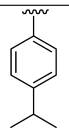
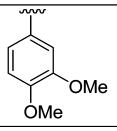
EXPERIMENTAL SECTION

Biology. LN229-HRE-luciferase glioblastoma cells were used to perform the assay. These cells contain a stably integrated reporter construct (pBI-GL HRE V6R plasmid) made of six copies of the HIF responsive element derived from the *VEGF* gene as previously described.[26]The 48-well plates were seeded with 3×10^4 cells per well and incubated under normoxic conditions for 24 h. Cells were then pretreated with different concentrations of **1** or its analogues for 1 h and then transferred to hypoxic conditions. After 24 h, the medium was aspirated, cells were lysed, and reporter activity was measured in

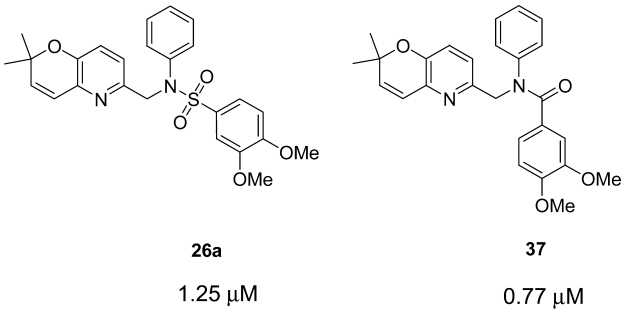
dx.doi.org/10.1021/jm201018g|J. Med. Chem. 2011, 54, 8471–8489

Table 9. Structures and Activities of Analogues 36[a]



Compound	R_1	R_2	IC_{50} (μM)
36a			1.4
36b			1.8
36c			5.8
36d			1.1

[a]Results were from single runs.



26a 1.25 μM **37** 0.77 μM

Figure 3. Comparison of structures and activities of **26a** and **37**.

the lysate using the luciferase assay system (Promega, Madison, WI) with a 20/20[n] luminometer (Promega).

Chemistry. General. All commercial chemicals and solvents were reagent grade and were used without further purification unless otherwise indicated. Microwave heating was performed in a single-mode microwave cavity of a Discover Synthesis System (CEM Corp.), and all microwave-irradiated reactions were conducted in heavy walled glass vials sealed with Teflon septa. ^{1}H NMR and ^{13}C NMR were recorded at 400 and 100 MHz, respectively, on a Bruker 400 NMR spectrometer with TMS or deuterated solvent as the internal standard. Coupling constants are in Hz. Mass spectral analysis was performed by the Mass Spectrometry Facilities at Georgia State University. The purities of tested compounds were assessed as being at least 95% with analytical HPLC, which was performed using a C18 5 μm (250 mm × 4.6 mm) column at 254 nm and with elution with a gradient of 70–80% solvent B (methanol) in solvent A (water) at 0.8 mL/min.

General Procedure for Reductive Amination for Synthesis of 2a–f. To a solution of 2,2-dimethyl-2*H*-chromene-6-carbaldehyde (1 equiv) in methanol was added the amine (2 equiv), sodium cyanoborohydride (2 equiv), and zinc chloride (anhydrous) (2 equiv). The mixture was stirred overnight. Then the solvent was removed by rotary evaporation and 1 M NaOH added to the residue. The organic layer was extracted with ethyl acetate or DCM (×2), dried over magnesium sulfate, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel).

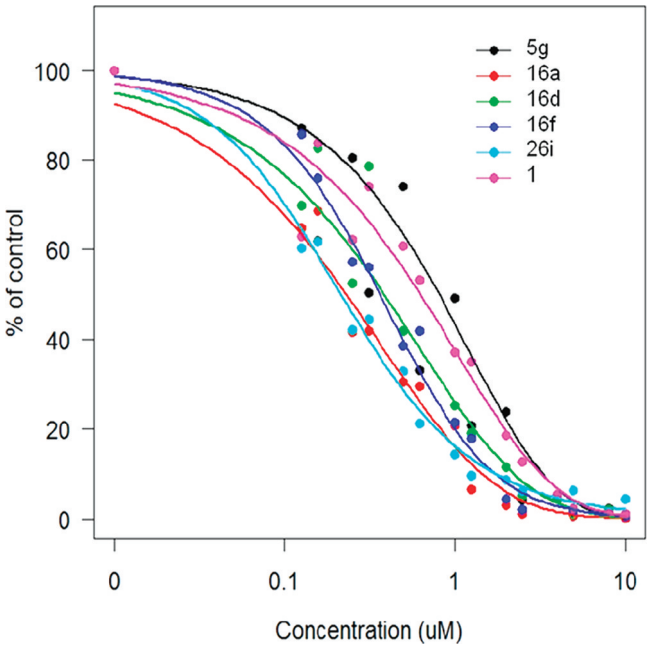


Figure 4. Dose response of a selected set of compounds using the HRE-luciferase reporter system. Cells were pretreated with various concentrations of inhibitors (control 1% DMSO) for 1 h in normoxia, followed by 24 h of incubation in hypoxia with inhibitors present. Luciferase activity was measured in cell extracts using a 20/20[n] luminometer (Turner Biosystems). Data, expressed as percent of control, are averages from at least four independent experiments carried out in triplicate.

(3,4-Dimethoxyphenyl)-(2,2-dimethyl-2*H*-chromen-6-ylmethyl)amine (2a). Yield: 60%. ^{1}H NMR ($CDCl_3$): δ 7.09 (dd, J = 8.2, 2.1 Hz, 1H), 6.99 (d, J = 2.0 Hz, 1H), 6.74 (d, J = 8.4 Hz, 2H), 6.30 (s, 1H), 6.30–6.24 (m, 1H), 6.17 (dd, J = 8.5, 2.6 Hz, 1H), 5.61 (d, J = 9.8 Hz, 1H), 4.15 (s, 2H), 3.81 (t, J = 6.2 Hz, 6H), 1.43 ppm (s, 6H). ^{13}C NMR ($CDCl_3$): δ 152.2, 150.0, 143.3, 141.6, 131.6, 131.1,

dx.doi.org/10.1021/jm201018g | J. Med. Chem. 2011, 54, 8471–8489

Table 10. Average IC_{50} (from *n* Independent Experiments in μM) of Selected Arylsulfonamide HIF-1 Pathway Inhibitors As Established in the HRE-Luciferase Assay

compd	no. of repeats	IC_{50}	std error	95% CI [a]
1	42	0. 648	0.044	0.562, 0.734
16a	6	0.306	0.06	0.187, 0.425
5g	6	0.813	0.141	0.533, 1.093
16d	6	0.478	0.105	0.270, 0.686
16f	6	0.378	0.062	0.255, 0.501
26i	30	0.280	0.022	0.237, 0.323

[a]CI: confidence interval.

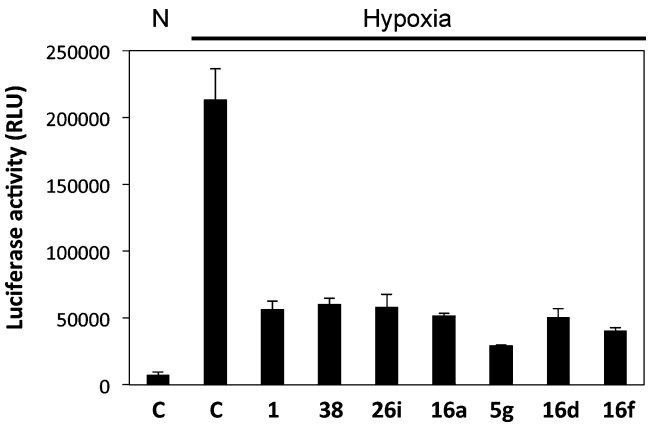


Figure 5. Luciferase reporter assays showing the effect of the selected set of compounds in LN229-VEGF-luc cells. Cells were pretreated with inhibitors (10 μM final concentration) for 1 h in normoxia, followed by 24 h of incubation in normoxia (N) or hypoxia (H) and luciferase measured as indicated in Figure 4. Each value represents an average from triplicates ± standard deviation. RLU = relative light units; C = vehicle control.

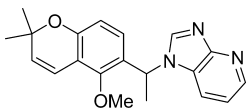


Figure 6. Structure of **38** (103D5).[33]

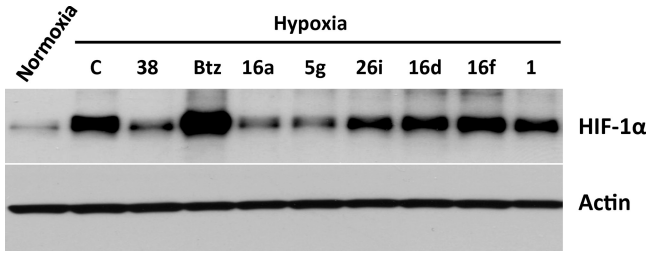


Figure 7. Western blots showing the effect of the selected set of compounds on hypoxic accumulation of HIF-1α in LN229 cells. Cells were pretreated with indicated inhibitors at 20 μM final concentration (Btz, bortezomib, 100 nM) for 1 h before incubation in normoxia or hypoxia for 24 h. Immunoblotting of HIF-1α and actin was as described earlier. C = vehicle control.[33]

128.4, 125.7, 122.2, 121.4, 116.4, 113.3, 103.6, 99.0, 76.2, 56.7, 55.7, 48.8, 28.1 ppm. HRMS (ESI) *m/z* calcd for $C_{20}H_{23}NO_3$ [(M + H)$^+$] 326.1756; found, 326.1750. HPLC: t_R = 9.41 min, 99.5%.

(2,2-Dimethyl-2*H*-chromen-6-ylmethyl)methylpyridin-2-yl-amine (2b). Yield: 60%. ^{1}H NMR ($CDCl_3$): δ 8.10–8.08 (m, 1H), 7.77–7.35 (m, 1H), 7.08–7.07 (m, 1H), 7.00–6.96 (m, 1H), 6.77–6.72 (m, 1H), 6.50–6.58 (m, 1H), 6.36 (d, *J* = 8.4 Hz, 1H), 6.28 (d, *J* = 9.6 Hz, 1H), 4.80 (s, br, 1H), 4.40 (s, 2H), 1.41 ppm (s, 6H). HRMS (ESI) *m/z* calcd for $C_{17}H_{18}N_2O$ [(M + H)$^+$] 267.149; found, 267.1505. HPLC: t_R = 12.8 min, 99.6%.

(2,2-Dimethyl-2*H*-chromen-6-ylmethyl)-(2,4-dimethylphenyl)amine (2c). Yield: 69%. ^{1}H NMR ($CDCl_3$): δ 7.10 (dd, *J* = 6.0 Hz, 2.4 Hz, 1H), 6.99 (d, *J* = 8.0 Hz), 6.92–6.90 (m, 1H), 6.74 (d, *J* = 8.4 Hz, 1H), 6.54 (d, *J* = 7.6 Hz, 1H), 6.30 (d, *J* = 9.6 Hz, 1H) 4.21 (s, 2H), 2.23 (s, 3H), 2.12 (s, 3H), 1.43 ppm (s, 6H). MS (ESI) *m/z* 292 [(M + H)$^+$]. HPLC: t_R = 20.84 min, 97.9%

4-[(2,2-Dimethyl-2*H*-chromen-6-ylmethyl)amino]benzoic Acid (2d). White powder. Yield: 58%. Mp 148 °C. ^{1}H NMR ($(CD_3)_2SO$): δ7.71 ppm (s, 2H), 7.08 (s, 1H), 7.03 (s, 1H), 6.68 (d, *J* = 8.2 Hz, 1H), 6.54 (d, *J* = 7.7 Hz, 2H), 6.37 (d, *J* = 9.8 Hz, 1H),5.72 (d, *J* = 9.8 Hz, 1H), 4.17 (d, *J* = 5.5 Hz, 2H), 1.35 (s, 6H). HRMS (ESI) *m/z* calcd for $C_{19}H_{19}NO_3$ [(M − H)$^+$] 308.1287; found, 308.1276.

(2-Bromophenyl)-(2,2-dimethyl-2*H*-chromen-6-ylmethyl)-amine (2e). Yield: 11%. ^{1}H NMR ($CDCl_3$): δ 7.43 (d, *J* = 7.8 Hz, 1H), 7.20–7.04 (m, 2H), 6.97 (s, 1H), 6.75 (d, *J* = 8.2 Hz, 1H), 6.62 (d, *J* = 8.0 Hz, 1H), 6.58 (d, *J* = 7.1 Hz, 1H), 6.30 (d, *J* = 9.8 Hz, 1H), 5.61 (d, *J* = 9.8 Hz, 1H), 4.63 (s, 1H), 4.26 (d, *J* = 5.3 Hz, 2H), 1.43 ppm (s, 6H). ^{13}C NMR ($CDCl_3$): δ 152.3, 144.9, 132.3, 131.1, 130.7, 128.5 128.1, 125.4, 122.2, 121.4, 117.9, 116.5, 111.6, 109.6, 76.3, 47.6, 28.0 ppm. HRMS (ESI) *m/z* calcd for $C_{18}H_{18}NOBr$ [(M + H)$^+$] 344.0650; found, 344.0663. HPLC: t_R = 19.71 min, 97.9%.

(2,2-Dimethyl-2*H*-chromen-6-ylmethyl)-(2-fluorophenyl)-amine (2f). Yield: 71%. ^{1}H NMR ($CDCl_3$): δ 7.10 (dd, *J* = 6.0, 2.0 Hz, 1H), 6.99–6.94 (m, 3H), 6.76–6.56 (m, 3H), 6.29 (d, *J* = 9.6 Hz, 1H), 5.61 (d, *J* = 9.6 Hz, 1H), 4.23 (s, 3H), 1.43 ppm (s, 6H). ^{13}C NMR ($CDCl_3$): δ 152.4, 143.9, 138.1, 136., 130.9, 128.3, 128.2, 127.5, 127.1, 124.7, 123.5, 122.3, 121.2, 116.2, 77.4, 28.1, 21.5 ppm. HRMS (ESI) *m/z* calcd for $C_{18}H_{18}NOF$ [(M + H)$^+$] 284.1451; found, 284.1442. HPLC: t_R = 16.55 min, 97.3%.

General Procedure for Synthesis of 3a, 3b, and 3c by Methylation of Secondary Amines 2a, 2b, and 2c, Respectively. A solution of secondary amine **2** (1 equiv) in THF was added to a flask containing NaH (2 equiv) in THF. After 5 min, MeI (2 equiv) was added and the mixture stirred overnight. The reaction mixture was quenched with water and diluted with ethyl acetate. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel).

(3,4-Dimethoxyphenyl)-(2,2-dimethyl-2*H*-chromen-6-ylmethyl)methylamine (3a). Yield: 60%. ^{1}H NMR ($CDCl_3$): δ 6.98 (dt, *J* = 7.2, 3.6 Hz, 1H), 6.87 (s, 1H), 6.78 (d, *J* = 8.7 Hz, 1H), 6.73 (s, 1H), 6.43 (s, 1H), 6.27 (d, *J* = 10.1 Hz, 2H), 5.59 (d, *J* = 9.8 Hz, 1H), 4.31 (s, 2H), 3.82 (d, *J* = 2.2 Hz, 6H), 2.89 (s, 3H), 1.42 ppm (s, 6H). ^{13}C NMR ($CDCl_3$): δ 149.7, 145.6, 131.2, 130.9, 127.9, 125.1, 122.3, 121.3, 116.3, 113.0, 104.8, 99.5, 77.4, 77.0, 76.7, 76.2, 57.6, 56.7, 55.8, 38.9, 28.0 ppm. HRMS (ESI) *m/z* calcd for $C_{21}H_{25}NO_3$ [(M + H)$^+$] 340.1913; found, 340.1900. HPLC: t_R = 21.7, 98.7%.

(2,2-Dimethyl-2*H*-chromen-6-ylmethyl)methylpyridin-2-yl-amine (3b). Yield: 81%. ^{1}H NMR ($CDCl_3$): δ 8.21 (m, 1H), 7.45 (m, 1H), 6.99 (d, *J* = 8.5 Hz, 1H), 6.86 (s, 1H), 6.72 (d, *J* = 8.0 Hz, 1H), 6.52–6.59 (m, 2H), 6.28 (d, *J* = 10 Hz, 1H), 5.60 (d, *J* = 10 Hz, 1H), 4.70 (s, 2H), 3.06 (s, 3H), 1.44 ppm (s, 6H). ^{13}C NMR ($CDCl_3$): δ 151.9, 148.0, 137.3, 130.9, 130.8, 127.8, 125.0, 122.4, 121.3, 116.3, 11.7, 105.8, 76.1, 52.6, 36.0, 28.0 ppm. HRMS (ESI) *m/z* calcd for $C_{18}H_{20}N_2O$ [M + H)$^+$] 281.1654; found, 281.1659. HPLC: t_R = 16.55, 97.3%.

(2,2-Dimethyl-2*H*-chromen-6-ylmethyl)-(2,4-dimethylphenyl)methylamine (3c). Yield: 48%. ^{1}H NMR ($CDCl_3$): δ 7.13 (dd, *J* = 2.0, 6.0 Hz, 1H), 7.05–7.00 (m, 4H), 6.75 (d, *J* = 8.0 Hz, 1H), 6.34 (d, *J* = 9.6 Hz, 1H), 5.63 (d, *J* = 9.2 Hz, 1H), 3.88 (s, 2H), 2.55 (s, 3H), 2,40 (s, 3H), 2.31 (s, 3H), 1.45 ppm (s, 6H). MS (ESI) *m/z* 308 [(M + H)$^+$]. HPLC: t_R = 12.17, 96.8%.

General Procedure for Synthesis of 5a–m by Alkyl Sulfonylation. To a solution of 2,2-dimethyl-2*H*-chromene-6-carbaldehyde (1 equiv) in methanol was added the primary amine (1 equiv), $ZnCl_2$ (2 equiv), and the mixture was stirred at room

dx.doi.org/10.1021/jm201018g | *J. Med. Chem.* 2011, 54, 8471–8489

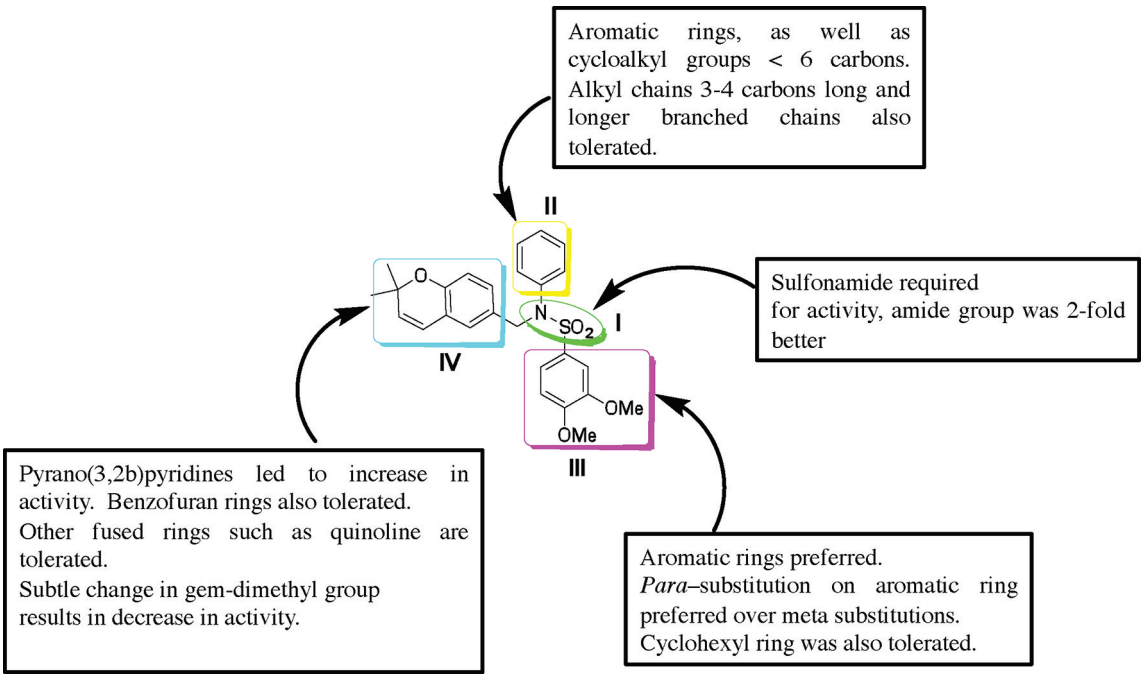


Figure 8. Structure–activity relationship of **1**.

temperature for 2 h. Then $NaCNBH_3$ (2 equiv) was added, and the mixture was stirred at room temperature overnight. The solvent was removed by rotary evaporation, and EtOAc was added to the residue. The solid was filtered through Celite and the filtrate washed with 1 M NaOH, water, and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude secondary amine product **4** was used without further purification.

To a solution of the secondary amine **4** (1 equiv) in DCM was added triethylamine (3 equiv) and the sulfonyl chloride (1.5 equiv). The mixture was stirred for 24–48 h. Then water was added and the organic layer extracted with DCM, dried over $MgSO_4$, concentrated under reduced pressure, and purified by flash column chromatography.

***N*-(2,2-Dimethyl-2*H*-chromen-6-ylmethyl)-*N*-isopropyl-3,4-dimethoxybenzenesulfonamide (5a).** Yield: 58%. Mp 132–134 °C. ^{1}H NMR ($CDCl_3$): δ 7.39 (dd, J = 6.4, 2.0 Hz, 1H), 7.22 (d, J = 2.0 Hz, 1H), 7.07 (dd, J = 6.0, 2.0 Hz, 1H), 7.00 (d, J = 2.0 Hz, 1H), 6.90 (t, J = 8.6 Hz, 1H), 6.69 (d, J = 8.2 Hz, 1H), 6.28 (d, J = 9.8 Hz, 1H), 5.60 (d, J = 9.8 Hz, 1H), 4.39–4.19 (m, 2H), 4.23–4.02 (m, 1H), 4.04–3.73 (m, 6H), 1.41 (s, 6H), 1.05 ppm (d, J = 7.2 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 152.2, 152.2, 149.0, 133.2, 131.0, 130.8, 128.6, 126.0, 122.3, 121.2, 120.8, 116.1, 110.5, 109.6, 76.3, 56.2, 56.1, 50.0, 46.0, 27.9, 21.3 ppm. HRMS (ESI) m/z calcd for $C_{23}H_{29}NO_5S$ [(M + Na)$^+$] 451.1664; found, 451.1651. HPLC: t_R = 11.76 min, 97.2%.

***N*-(2,2-Dimethyl-2*H*-chromen-6-ylmethyl)-3,4-dimethoxy-*N*-prop-2-ynylbenzenesulfonamide (5b).** Yield: 95%. ^{1}H NMR ($CDCl_3$): δ 7.53 (dd, J = 2.0 Hz, 1H), 7.38 (d, J = 2.0 Hz, 1H), 7.09–7.07 (m, 1H), 7.00–6.96 (m, 2H), 6.74 (d, J = 8.0 Hz, 1H), 6.31 (d, J = 9.6 Hz, 1H), 5.64 (d, J = 9.6 Hz, 1H), 4.24 (s, 1H), 4.01–3.96 (m, 7 H), 1.59 (s, 2H), 1.43 ppm (s, 6H). HRMS (ESI) m/z calcd for $C_{23}H_{25}NO_5S$ [(M + Na)$^+$] 450.1351; found, 450.1352. HPLC: t_R = 10.65 min, 96.61%.

(*N*-Butyl-*N*-(2,2-dimethyl-2*H*-chromen-6-ylmethyl)-3,4-dimethoxybenzenesulfonamide (5c). Yield: 55%. Mp 82 °C. ^{1}H NMR ($CDCl_3$): δ 7.46 (dd, J = 6.4, 2.1 Hz, 1H), 7.28 (m, 1H), 7.00–6.89 (m, 2H), 6.71 (d, J = 8.0 Hz, 1H), 6.27 (d, J = 10.0 Hz, 1H), 5.63 (d, J = 9.6 Hz, 1H), 4.23 (s, 1H), 3.97–3.92 (m, 6H), 3.10 (t, J = 7.6 Hz, 2H), 1.43(s, 6H), 1.38–1.16 (m, 6H), 0.79 (t, J = 7.6 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 152.3, 149.0, 132.2, 131.1, 129.1, 128.5, 126.4, 122.1, 121.3, 121.0, 116.2, 110.6, 109.8, 76.3, 56.2, 56.2, 51.1, 47.4, 30.0, 27.9, 19.9, 13.6 ppm. HRMS (ESI) m/z calcd for $C_{24}H_{31}NO_5S$ + Na 468.1821; found, 468.1815. HPLC: t_R = 14.0 min, 96.3%.

***N*-*tert*-Butyl-*N*-(2,2-Dimethyl-2*H*-chromen-6-ylmethyl)-3,4-dimethoxybenzenesulfonamide (5d).** Yield: 49%. ^{1}H NMR ($CDCl_3$): δ 7.41 (dd, J = 8.5, 2.1 Hz, 1H), 7.22–7.12 (m, 2H), 7.06 (d, J = 2.1 Hz, 1H), 6.87 (d, J = 8.5 Hz, 1H), 6.74 (t, J = 9.7 Hz, 1H), 6.30 (t, J = 8.9 Hz, 1H), 5.62 (t, J = 9.5 Hz, 1H), 4.56 (s, 2H), 3.94 (d, J = 13.4 Hz, 3H), 3.85 (d, J = 14.6 Hz, 3H), 1.48–1.39 (m, 6H), 1.33 ppm (s, 9H). MS (ESI) m/z 468 [(M + Na)$^+$]. HPLC: t_R = 13.2 min, 96.3%.

***N*-Allyl-*N*-(2,2-dimethyl-2*H*-chromen-6-ylmethyl)-3,4-dimethoxybenzenesulfonamide (5e).** Yield: 53%. Mp 94–98 °C. ^{1}H NMR ($CDCl_3$): δ 7.48 (dd, J = 8.4, 2.2 Hz, 1H), 7.29 (t, J = 2.3 Hz, 1H), 7.01–6.91 (m, 2H), 6.88 (d, J = 2.1 Hz, 1H), 6.72 (t, J = 9.7 Hz, 1H), 6.29 (d, J = 6.4 Hz, 1H), 5.64 (t, J = 10.6 Hz, 1H), 5.53 (ddt, J = 16.7, 10.2, 6.5 Hz, 1H), 5.09 (ddd, J = 18.4, 13.6, 1.3 Hz, 2H), 4.28 (d, J = 25.4 Hz, 2H), 4.03–3.95 (m, 3H), 3.95–3.88 (m, 3H), 3.84–3.72 (m, 2H), 1.44 ppm (s, 6H). ^{13}C NMR ($CDCl_3$): δ 152.6, 152.4, 149.1, 132.4, 132.4, 131.9, 129.4, 127.9, 126.6, 122.1, 121.3, 121.1, 199.2, 116.2, 110.6, 109.8, 76.4, 56.2, 56.18, 49.6, 49.2, 28.0 ppm. EI probe: M$^+$ 429. HPLC: t_R = 11.9 min, 97.0%

***N*-(2,2-Dimethyl-2*H*-chromen-6-ylmethyl)-3,4-dimethoxy-*N*-(3-methylbutyl)benzenesulfonamide (5f).** Yield: 31%. Mp 103–105 °C. ^{1}H NMR ($CDCl_3$): δ 7.44 (dd, J = 6.4, 2.0 Hz, 1H), 7.29–7.28 (m, 1H), 6.96–6.94 (m, 2H), 6.83 (s, 1H), 6.24 (d, J = 10.0 Hz, 1H), 5.62 (d, J = 10.0 Hz, 1H), 4.23 (s, 2H), 3.97(s, 3H), 3.92 (s, 3H), 2.90 (d, J = 7.6 Hz, 2H), 1.75 (sep, J = 6.8 Hz, 1H), 1.43 (s, 6H),1.28 (s, 2H), 0.792–0.779 ppm (m, 6H). ^{13}C NMR ($CDCl_3$): δ 152.5, 152.3, 149.0, 132.1, 131.1, 129.2, 128.5, 126.5, 122.1, 121.3 121.1, 116.2, 110.5, 109.9, 76.3, 56.2, 56.2, 55.8, 52.1, 27.9, 26.9, 20.0 ppm. HRMS (ESI) m/z calcd for $C_{24}H_{31}NO_5S$ 468.1821 [(M + Na)$^+$]; found, 468.1801. HPLC: t_R = 13.92 min, 97.5%.

***N*-Cyclopentyl-*N*-(2,2-dimethyl-2*H*-chromen-6-ylmethyl)-3,4-dimethoxybenzenesulfonamide (5g).** Yield: 58%. Mp 110–112 °C. ^{1}H NMR ($CDCl_3$): δ 7.39 (d, J = 6.4 Hz, 1H), 7.21 (d, J = 2.1 Hz, 1H), 7.10–6.82 (m, 3H), 6.67 (d, J = 8.2 Hz, 1H), 6.26 (d, J = 9.8 Hz, 1H), 5.57 (d, J = 9.8 Hz, 1H), 4.22 (s, 2H), 3.88 (d, J = 20.2 Hz, 6H), 1.70–1.18 (m, 15H). ^{13}C NMR ($CDCl_3$): δ 152.2, 152.1, 150.0, 132.7, 131.0, 130.9, 127.9, 152.3, 122.3, 121.2, 121.0, 116.1, 110.5, 109.8, 76.2, 59.5, 56.2, 56.1, 46.8, 29.3, 28.0, 23.5 ppm. HRMS (ESI) m/z calcd for $C_{25}H_{31}NO_5S$ 480.1842 [(M + Na)$^+$]; found, 480.1822. HPLC: t_R = 14.11 min, 98.1%.

***N*-Cyclopropyl-*N*-(2,2-dimethyl-2*H*-chromen-6-ylmethyl)-3,4-dimethoxybenzenesulfonamide (5h).** Off-white semisolid.

dx.doi.org/10.1021/jm201018g | *J. Med. Chem.* 2011, 54, 8471–8489

Yield: 47%. Mp 94 °C. ^{1}H NMR ($CDCl_3$): δ 7.46 (dd, J = 2.0, 6.4 Hz, 1H), 7.27 (d, J = 2.0 Hz, 1H), 7.06 (dd, J = 2.0, 6.4 Hz, 1H), 6.97−6.93 (m, 2H), 6.70 (d, J = 8.0 Hz, 1H), 6.29 (d, J = 9.6 Hz, 1H), 4.27 (s, 2H), 3.94 (s, 3H), 3.90 (s, 3H), 5.62 (d, J = 9.6 Hz, 1H), 2.01 (quin, J = 4.0 Hz, 1H), 1.44 (s, 6H), 0.72 (q, J = 3.2 Hz, 2H), 0.59 (q, J = 3.2 Hz, 2H). ^{13}C NMR ($CDCl_3$): δ 152.5, 148.9, 131.0, 130.5, 129.7, 129.0, 126.9, 122.2, 121.6, 121.1, 116.0, 110.4, 110.2, 76.3, 56.2, 56.2, 54.2, 30.6, 28.0, 27.3 ppm. HRMS (ESI) m/z calcd for $C_{23}H_{27}NO_5S$ 452.1508 [(M + Na)$^+$]; found, 452.1489. HPLC: t_R = 7.48 min, 99.5%.

***N*-Cyclohexyl-*N*-(2,2-dimethyl-2*H*-chromen-6-ylmethyl)-3,4-dimethoxybenzenesulfonamide (5i).** Yield: 79%. ^{1}H NMR ($CDCl_3$): δ 7.42 (dd, J = 2.0 Hz, 6.4 Hz, 1H), 7.30−7.15 (m, 1H), 7.16 (d, J = 8.4 Hz, 1H), 7.10−7.08 (m, 1H), 7.02 (d, J = 2.0 Hz, 1H), 6.91 (d, J = 8.4 Hz, 1H), 6.71 (d, J = 8.0 Hz, 1H), 6.31 (d, J = 9.6 Hz, 1H), 5.63 (d, J = 9.6 Hz, 1H), 4.31 (s, 2H), 3.95 (s, 3H), 3.90 (s, 3H), 1.70−1.54 (m, 4H), 1.43 (s, 6H), 1.27−1.20 ppm (m, 6H). Yield: 79%. HPLC: t_R = 14.4 min, 99.5%.

General Procedure for the Synthesis of 5j−m. To a solution of secondary amine **4** (1 equiv) in DCM were added triethylamine (3 equiv) and then the appropriate sulfonyl chloride (2 equiv). The mixture was stirred at room temperature for 24 h. Then saturated NH_4Cl was added to the reaction mixture, which was extracted with DCM (×2). After drying over $MgSO_4$, the DCM solution was concentrated under vacuum. The crude product was purified by flash column chromatography (silica gel).

***N*-((2,2-Dimethyl-2*H*-chromen-6-yl)methyl)-4-methoxy-*N*-phenylbenzenesulfonamide (5j).** Yield: 26.4 mg (30%). ^{1}H NMR ($CDCl_3$): δ 7.60 (d, J = 9.2 Hz, 2H), 7.28−7.22 (m, 3H), 7.00−6.93 (m, 4H), 6.91−6.88 (m, 2H), 6.62 (s, 1H), 6.24 (d, J = 10.0 Hz, 1H), 5.58 (d, J = 10 Hz, 1H), 4.62 (s, 2H), 3.90 (s, 3H), 1.40 ppm (s, 6H). ^{13}C NMR ($CDCl_3$): δ 162.9, 139.1, 130.9, 129.8, 129.3, 129.1, 128.8, 128.1, 127.8, 126.6, 122.3, 116.0, 114.0, 76.3, 55.6, 54.3, 28.0 ppm. HRMS (ESI) m/z calcd for $C_{23}H_{27}NO_5S$ 452.1508 [(M + H)$^+$]; found, 452.1489. HPLC: t_R = 14.03 min, 96.1%.

***N*-((2,2-Dimethyl-2*H*-chromen-6-yl)methyl)-3,5-dimethyl-*N*-phenylbenzenesulfonamide (5k).** Yield: 65 mg, (40%). ^{1}H NMR: δ 7.54 (s, 2H), 7.30−7.23 (m, 3H), 7.00−6.97 (m, 1H), 6.90−6.88 (m, 2H), 6.60 (d, J = 8.8 Hz, 1H), 6.24 (d, J = 10 Hz, 1H), 5.58 (d, J = 9.6 Hz, 1H), 4.63 (s, 2H), 2.41 (s, 3H), 2.36 (s, 3H), 1.42(s, 6H). MS (ESI) m/z 458 [(M + Na)$^+$]. HPLC: t_R = 23.7 min, 96.8%.

2,5-Dichloro-*N*-((2,2-dimethyl-2*H*-chromen-6-yl)methyl)-*N*-phenylbenzenesulfonamide (5l). Yield: 69 mg (32%). ^{1}H NMR ($CDCl_3$): δ 7.84 (d, J = 2.4 Hz, 1H), 7.48 (d, J = 8.4 Hz, 1H), 7.44−7.40 (m, 1H), 7.23−7.21 (m, 3H), 7.06−7.04 (m, 2H), 6.92−6.90 (m, 2H), 6.64 (d, J = 7.6 Hz, 1H), 6.27 (d, J = 10.0 Hz, 1H), 5.60 (d, J = 9.6 Hz, 1H), 4.92 (s, 2H), 1.42 ppm (s, 6H). MS (ESI) m/z 471 [(M + Na)$^+$].

4,4-Bromo-*N*-((2,2-dimethyl-2*H*-chromen-6-yl)methyl)-*N*-phenyl-2-(trifluoromethoxy)benzenesulfonamide (5m). Yield: 23%. ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 6H), 4.95 (s, 2H), 5.60 (d, 1H, J = 9.6), 6.27 (d, 1H, J = 10), 6.64 (s, 1H, J = 7.6), 6.91 (m, 2H), 7.05 (m, 2H), 7.21 (m, 3H,) 7.43 (m, 1H) 7.48 (m, 1H), 7.84 ppm (d, 1H, 2.4). HPLC: t_R = 19.6 min, 96.7%.

4-(3-Methylpent-1-yn-3-yloxy)benzaldehyde (7). To a solution of 3-methyl-1-pentyn-3-ol **6** (0.319 mL, 2.83 mmol) in acetonitrile (3 mL) at 0 °C was added DBU (0.55 mL, 3.69 mmol). Then TFAA (0.34 mL, 2.46 mmol) was added dropwise and the solution was stirred at 0 °C for 30 min. To a solution of 4-hydroxybenzaldehyde (300 mg, 2.46 mmol) in acetonitrile at 0 °C were added DBU (0.55 mL 3.69 mmol) and $CuCl_2{\cdot}2H_2O$ (0.42 mg, 0.0025 mmol). The first mixture was added to the second mixture over a period of 5 min. The mixture was stirred overnight. The solvent was removed by rotary evaporation and the residue diluted with DCM. Then the organic layer washed with 1 M HCl, 1 M NaOH, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuum to give 170 mg (30%) of product. ^{1}H NMR ($CDCl_3$): δ 9.91(s, 1H), 7.83−7.81 (m, 2H), 7.36−7.34 (m, 2H), 2.69 (s, 1H), 2.06−1.92 (m, 2H), 1.66 (s, 3H), 1.12 ppm (t, J = 7.2 Hz, 3H).

2-Ethyl-2-methyl-2*H*-chromene-6-carbaldehyde (8). A solution of **7** (170 mg) in xylene (3 mL) was subjected to microwave irradiation for 100 min at 220 W, 200 Torr, 120 °C. The solvent was removed in vacuum to give a quantitative yield of the product (170 mg). ^{1}H NMR ($CDCl_3$): δ 9.81 (s, 1H), 7.63 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 7.50 (d, J = 2.0 Hz, 1H), 6.85 (d, J = 8.4 Hz, 1H), 6.42 (d, J = 10.0 Hz, 1H), 5.62 (d, J = 10.0 Hz, 1H), 1.81−1.66 (m, 3H), 1.43 (s, 3H), 0.97 ppm (t, J = 7.6 Hz, 3H).

***N*-((2-Ethyl-2-methyl-2*H*-chromen-6-yl)methyl)benzenamine (9).** Reaction was carried out following the same procedure as for **2a−f** using 170 mg of **8** to give 90.9 mg (41%) of product. ^{1}H NMR ($CDCl_3$): δ 7.22−7.18 (m, 5H), 7.11 (dd, J = 6.0 Hz, 2.4 Hz, 1H), 6.99 (d, J = 2.0 Hz, 2H), 6.77 −6.74 (m, 3H), 6.68−6.66 (m, 2H), 6.36 (d, J = 10.0 Hz, 1H), 5.58 (d, J = 10.0 Hz, 1H), 4.21 (s, 2H), 1.76−1.71 (m, 3H), 1.33(s, 3H), 0.96 ppm (t, J = 7.6 Hz, 3H).

***N*-((2-Ethyl-2-methyl-2*H*-chromen-6-yl)methyl)-3,4-dimethoxy-*N*-phenylbenzenesulfonamide (10).** To a solution of **9** (80 mg, 0.29 mmol) in DCM (3 mL) were added Et_3N (0.12 mL, 0.85 mmol) and 3,4-dimethoxybenzenesulfonyl chloride (135 mg, 0.573 mmol). After 24 h, saturated NH_4Cl was added to the reaction mixture and the aqueous layer was extracted with DCM (5 × 2 mL). The organic layers was combined, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by column (silica gel, 5:1 hexane/EtOAc) to give a white solid (42.6 mg). Yield: 32%. Mp 129−130 °C. ^{1}H NMR ($CDCl_3$): δ 7.35 (dd, J = 6.4 Hz, 2.0 Hz, 1H), 7.25−7.23 (m, 3H), 7.02−6.98 (m, 3H), 6.94 (d, J = 8.4 Hz, 1H), 6.91−6.88 (m, 2H), 6.59 (d, J = 8 Hz, 1H), 6.28 (d, J = 10 Hz, 1H), 5.53 (d, J = 10 Hz, 1H) 4.62 (s, 2H), 3.98 (s, 3H), 3.77 (s, 3H), 1.71−1.65 (m, 2H), 1.43 (s, 3H), 0.94 ppm (t, J = 7.6 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 152.8, 152.5, 148.7, 139.2, 130.5, 129.8, 129.3, 129.2, 128.8, 127.9, 127.8, 126.6, 122.8, 121.4, 121.1, 115.8, 110.4, 79.0, 56.2, 56.1, 54.3, 34.0, 26.0 ppm. MS (ESI) m/z 502 [(M + Na)$^+$]. HPLC: t_R = 5.9 min, 98.9%.

***N*-Phenylquinolin-3-amine (12).** To a solution of quinoline-3-carbaldehyde **11** (79 mg, 0.5 mmol) in MeOH (5 mL) were added aniline (0.05 mL, 0.55 mmol) and $ZnCl_2$ (136 mg, 2.0 mmol), and the mixture was stirred at room temperature for 15 min. Then $NaCNBH_3$ (62.8 mg, 2.0 mmol) was added and the mixture was stirred overnight at room temperature. The solvent was removed by rotary evaporation and the residue suspended in EtOAc. The organic layers were combined and washed with $NaHCO_3$, water, and brine and then dried over $MgSO_4$. Concentration in vacuo gave the crude product, which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 9.05 (s, 1H), 8.23 (d, J = 7.6 Hz, 2H), 7.77 (d, J = 8.0 Hz, 1H), 7.71−7.66 (m, 1H), 7.54−7.51 (m, 1H), 7.21−7.16 (m, 2H), 6.76−6.72 (m, 1H), 6.68−6.60 (m, 2H), 4.54 (s, 2H), 4.16 (s, br, 1H).

3,4-Dimethoxy-*N*-phenyl-*N*-(quinolin-3-ylmethyl)-benzenesulfonamide (13). To a solution of **12** (75 mg, 0.320 mmol) in DCM was added 3,4-dimethoxybenzylsulfonyl chloride (83 mg, 0.35 mmol) and triethylamine (0.09 mL, 0.640 mmol). The mixture was stirred overnight at room temperature, and the reaction mixture was washed with water and brine. The organic layer was dried over $MgSO_4$, concentrated in vacuo, and purified by column chromatography (silica gel, 2:1 hexane/EtOAc) to give a white powder. Yield: 45%. Mp 173. ^{1}H NMR ($CDCl_3$): 8.86 (s, 1H), 8.14(d, J = 8.0 Hz, 2H), 7.84 (dd, J = 7.2 Hz, 1.2 Hz, 1H), 7.80−7.76 (m, 1H), 7.64 −7.60 (m, 1H), 7.49 (dd, J = 6.0 Hz, 2.4 Hz, 1H), 7.38−7.30 (m, 3H), 7.17−7.14 (m, 2H), 7.08−7.05 (m, 2H), 5.03 (s, 2H), 4.08 (s, 3H), 3.85 ppm (s, 3H). ^{13}C NMR ($CDCl_3$): 152.8, 150.8, 148.8, 147.6, 138.0 135.7, 129.6, 129.2, 129.1, 129.0, 128.2, 127.7, 127.7, 126.9, 121.6, 110.5, 110.4, 56.2, 56.1, 52.4 ppm. MS (ESI) m/z 435 [(M + H)$^+$]. HPLC: t_R = 14.6 min, 95.2%.

***N*-((2,2-Dimethyl-2*H*-chromen-6-yl)methyl)benzenamine (15a).** To a solution of 2,3-dihydro-2,2-dimethylbenzofuran-5-carboxaldehyde **14** (250 mg, 1.42 mmol) in MeOH (10 mL) were added aniline (0.14 mL, 1.022 mmol), $NaCNBH_3$ (178 mg, 2.84 mmol), and $ZnCl_2$ (dried in oven) (387 mg, 2.84 mmol). The mixture was stirred at room temperature overnight, and then the solvent was removed by rotary evaporation. Then 0.1 M NaOH was added to the resulting residue, which was extracted with EtOAc (×2). The combined organic

layers were dried over $MgSO_4$ and concentrated in vacuum to give 301 mg of the product (84%). ^{1}H NMR ($CDCl_3$): δ 7.29−7.22 (m, 3H), 7.17 (d, J = 8.0 Hz, 1H), 6.95−6.70 (m, 4H), 4.28 (s, 2H), 3.06 (s, 2H), 1.55 ppm (s, 6H).

***N*-((2,2-Dimethyl-2,3-dihydrobenzofuran-5-yl)methyl)-3,4-dimethoxy-*N*-phenylbenzenesulfonamide (16a).** To a solution of **15a** (100 mg, 0.395 mmol) in DCM (5 mL) were added triethylamine (0.17 mL, 0.790 mmol) and 3,4-dimethoxybenzenesulfonyl chloride (187 mg, 0.790 mmol) dissolved in 1 mL of DCM, and the mixture was stirred for 72 h. Ammonium chloride was added to the reaction mixture, which was then extracted with DCM (×2). After drying of the combined organic layers over $MgSO_4$ and concentration in vacuum, the crude reaction mixture was purified by column (silica gel, 3:1 hexane/EtOAc) to give a white solid (76 mg, 42%). Mp 136 °C. ^{1}H NMR ($CDCl_3$): δ 7.33 (dd, J = 2.13, 8.44, 1H), 7.26−7.21 (m, 3H), 7.09 (s, 1H) 7.00−6.96 (m, 3H), 6.92 (d, J = 8.5, 1H), 6.83 (d, J = 8.1,1H), 6.52 (d, J = 8.1, 1H), 4.62 (s, 2H), 3.95 (s, 1H), 3.75 (s, 1H), 2.92 (s, 2H), 1.42 ppm (s, 1H). ^{13}C NMR ($CDCl_3$): δ 158.4, 152.5, 148.7, 139.3, 129.2, 127.5, 125.6, 121.4, 110.4, 108.9, 86.9, 56.2, 56.1, 54.6, 42.7, 28.2 ppm. MS (ESI) m/z 435 [(M + H)]. HPLC: t_R = 10.5 min, 96.1%.

General Procedure for the Synthesis of Compounds 16b−f. To a solution of **14** in methanol was added amine (1 0.1 equiv) and zinc chloride (2 equiv), and the reaction mixture was stirred for 2 h before $NaCNBH_3$ (2 equiv) was added. The mixture was then stirred at room temperature overnight. The solvent was removed by rotary evaporation and the residue diluted with EtOAc and washed with Na_2CO_3 (saturated) and brine. The organic layers were dried over $MgSO_4$ and concentrated in vacuo. The product was used without further purification in the next step. To the resulting secondary amine **15** (1 equiv) in DCM was added Et_3N (2 equiv) and the appropriate aryl- or alkylsulfonyl chloride (1.1 equiv), and the mixture was stirred at room temperature overnight. The reaction mixture was diluted with DCM and washed with water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography (silica gel).

***N*-Cycloheptyl-*N*-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)methyl)-3,4-dimethoxybenzenesulfonamide (16b).** Yield: 14%. Mp 90 °C. ^{1}H NMR ($CDCl_3$): δ 7.43 (dd, J = 2.14, 8.44, 1H), 7.25−7.24 (m, 2H), 7.05 (d, J = 8.13, 1H), 6.92 (d, J = 8.5, 1H), 6.65 (d, J = 8.1, 1H), 4.28 (s, 2H), 3.96 (s, 3H), 3.90 (s, 3H), 3.00 (s, 2H), 1.63−1.51 (m, 8H), 1.48 (s, 6H), 1.45−1.27 ppm (m, 7H). HRMS (ESI) calcd for $C_{26}H_{35}NO_5S$ m/z [(M + Na)$^+$] 496.2134; found, 496.2122. HPLC: t_R = 18.6 min, 99.4%.

***N*-((2,2-Dimethyl-2,3-dihydrobenzofuran-5-yl)methyl)-*N*-isopropyl-3,4-dimethoxybenzenesulfonamide (16c).** Yield: 18%. ^{1}H NMR ($CDCl_3$): δ 7.45 (d, J = 8.2 Hz, 1H), 7.27 (s, 1H), 7.10 (d, J = 15.6 Hz, 1H), 6.94 (t, J = 7.5 Hz, 2H), 6.63 (d, J = 7.6 Hz, 1H), 4.24 (s, 2H), 3.94 (d, J = 19.3 Hz, 6H), 3.05−2.93 (m, 2H), 2.91 (d, J = 6.9 Hz, 2H), 1.82−1.66 (m, 1H), 1.48 (s, 6H), 0.77 ppm (d, J = 5.7 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 158.5, 152.3, 149.0, 132.1, 128.4, 127.9, 127.7, 125.6, 121.1, 110.5, 109.9, 109.0, 87.1, 77.4, 77.1, 76.7, 56.2, 56.1, 55.8, 52.4, 42.7, 28.1, 26.9, 20.0 ppm. HRMS (ESI) m/z calcd for $C_{23}H_{31}NO_5S$ [(M + Na)$^+$] 456.1821; found, 456.1833. HPLC: t_R = 11.5 min, 97.6%.

***N*-Butyl-*N*-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)-methyl)-3,4-dimethoxybenzenesulfonamide (16d).** Yield: 21%. ^{1}H NMR ($CDCl_3$): δ 7.47 (dd, J = 8.4, 1.5 Hz, 1H), 7.34−7.22 (m, 2H), 7.13 (s, 1H), 6.96 (d, J = 8.4 Hz, 2H), 6.65 (d, J = 8.1 Hz, 1H), 4.25 (s, 2H), 3.95 (d, J = 16.7 Hz, 6H), 3.22−3.03 (m, 2H), 2.99 (s, 2H), 1.57 (d, J = 21.8 Hz, 1H), 1.49 (s, 6H), 1.41−1.24 (m, 3H), 1.23−1.08 (m, 2H), 0.79 ppm (t, J = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 158.6, 152.2, 149.2, 143.0, 132.3, 128.3, 127.4, 125.5, 120.9, 110.5, 109.8, 109.0, 87.1, 77.4, 77.0, 76.7, 56.2, 56.1, 51.3, 47.3, 42.7, 29.9, 28.1, 19.9, 13.7 ppm. HRMS (ESI) m/z calcd for $C_{23}H_{31}NO_5S$ [(M + Na)$^+$] 456.1821; found, 456.1812. HPLC: t_R = 11.2 min, 98.0%.

***N*-Cyclohexyl-*N*-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)-methyl)-3,4-dimethoxybenzenesulfonamide (16e).** Yield: 36%. Mp 102 °C. ^{1}H NMR ($CDCl_3$) δ 7.41 (d, J = 8.4, 1H), 7.22 (s, 2H), 7.03 (d, J = 7.9, 1H), 6.90 (d, J = 8.3, 1H), 6.63 (d, J = 7.9, 1H), 4.31 (s, 2H), 3.70 (1H) 3.94 (s, 3H) 3.88 (s, 3H), 2.98 (s, 1H), 1.69−1.52 (m, 7H), 1.47(s, 6H), 1.27−1.22 ppm (m, 4H). HRMS (ESI) m/z calcd for $C_{25}H_{33}$ NO_5S [(M + Na)$^+$] 482.1977; found, 482.1981. HPLC: t_R = 16.4 min, 95.6%.

***N*-Cyclopentyl-*N*-((2,2-dimethyl-2,3-dihydrobenzofuran-5-yl)methyl)-3,4-dimethoxybenzenesulfonamide (16f).** Yield: 31%. ^{1}H NMR ($CDCl_3$): δ 7.44 (d, J = 8.5, 1H), 7.27 (s, 2H), 7.04 (d, J = 8.10, 1H), 6.92 (d, J = 8.4, 1H), 6.65 (d, J = 8.1, 1H), 4.29 (s, 3H), 3.95 (s, 3H), 3.90 (s, 3H), 2.99 (s, 2H), 1.85−1.58 (m, 3H), 1.60−1.22 ppm (m, 12 H). HRMS (ESI) m/z calcd for $C_{24}H_{31}NO_5S$ [(M + Na)$^+$] 468.1821; found, 468.1817. HPLC: t_R = 14.4 min, 95.0%

2,2-Dimethyl-2*H*-pyrano[2,3-*b*]pyridine-6-carbaldehyde (18). To a solution of **17** (100 mg, 0.390 mmol) in dry ether (2 mL) was added BuLi (0.25 mL, 2.5 M solution in THF) dropwise at −65 °C, and the mixture was stirred for 15 min. Then DMF (anhydrous) was added dropwise and the mixture was stirred at −65 °C for 1.5 h. Water was added to quench the reaction, which was extracted with EtOAc (×2). The organic layers were washed with water (×1), brine (×1), dried over $MgSO_4$, and concentrated in vacuo to give a yellow oil. Purification by column chromatography (silica gel) with 6:1 hexane/EtOAc gave a white solid. Yield: 23 mg (31%). ^{1}H NMR ($CDCl_3$): δ 1.58 (s, 1H), 5.79 (d, 1H, J = 8.0 Hz), 6.36 (d, 1H, J = 9.6 Hz), 7.76 (s, 1H), 8.50 (s, 1H), 9.92 ppm (s, 1H).

***N*-((2,2-Dimethyl-2*H*-pyrano[2,3-*b*]pyridin-6-yl)methyl)-benzenamine (19a).** To a solution of 2,2-dimethyl-2*H*-pyrano[2,3-*b*]-pyridine-6-carbaldehyde **18** (20 mg, 0.106 mg) in methanol (1 mL) were added aniline (0.01 mL, 0.12 mmol), $NaCNBH_3$ (13 mg, 0.212 mmol), and $ZnCl_2$ (29 mg, 0.212 mmol). The mixture was stirred for 30 min, after which the solvent was removed by rotary evaporation and the 1 M NaOH added to the residue, extracted with DCM, dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography (3:1 hexane/EtOAc) gave a white solid. Yield: 20 mg (72%). ^{1}H NMR ($CDCl_3$) δ 8.01 (s, 1H), 7.29 (s, 1H), 7.26−7.16 (m, 2H), 6.74 (t, J = 7.2 Hz, 1H), 6.63 (d, J = 8.0 Hz, 1H), 6.26 (d, J = 9.6 Hz, 1H), 5.67 (d, J = 9.6 Hz, 1H), 4.21 (s, 2H), 1.51 ppm (s, 6H). ^{13}C NMR ($CDCl_3$) δ 159.6, 147.8, 146.4, 133.8, 132.2, 129.3, 128.4, 120.9, 118.0, 115.4, 113.0, 79.2, 45.4, 28.8 ppm. HRMS (ESI) m/z calcd for $C_{11}H_{11}NO_2$ [(M + H)$^+$] 190.0868; found, 190.0870.

***N*-((2,2-Dimethyl-2*H*-pyrano[2,3-*b*]pyridin-6-yl)methyl)-cyclohexanamine (19b).** To a solution of 2,2-dimethyl-2*H*-pyrano-[2,3-*b*]pyridine-6-carbaldehyde **18** (23 mg, 0.121 mmol) in MeOH (1 mL) were added cyclohexylamine (0.014 mL, 0.121 mmol), $NaCNBH_3$ (15 mg, 0.242 mmol), and zinc chloride (33 mg, 0.242 mmol), and the mixture was stirred overnight . The solvent was removed by rotary evaporation and the residue dissolved in EtOAc and washed with 1 M NaOH, water, and brine, dried over $MgSO_4$, and concentrated in vacuo. The product was used in the next step without further purification.

***N*-((2,2-Dimethyl-2*H*-pyrano[2,3-*b*]pyridin-6-yl)methyl)-3,4-dimethoxy-*N*-phenylbenzenesulfonamide (20a).** To a solution of **19a** (20 mg, 0.075 mmol) in DCM (1 mL) were added 3,4-dimethoxybenzenesulfonyl chloride (36 mg, 0.150 mmol) and triethylamine (0.021 mL, 0.150 mmol). The mixture was stirred for 24 h at room temperature. The reaction mixture was washed with water (×2) and the organic layer dried over $MgSO_4$ and concentrated in vacuo. Column chromatography (2:1 hexane/EtOAc) gave a white solid (15 mg). Yield: 43%. ^{1}H NMR ($CDCl_3$): δ 1.47(s, 6H), 3.76 (s, 3H), 3.97 (s, 3H), 4.60 (s, 2H), 5.66 (d,1H, J = 9.6), 6.26 (d, 1H, J = 10), 6.93−6.99 (m, 4H), 7.23−7.25 (m, 3H), 7.32−7.36 (m, 2H), 7.63 ppm (d, 1H, J = 2.4). HRMS (ESI) m/z calcd for $C_{25}H_{25}N_2O_5S$ [M + H)$^+$] 467.1641; found, 467.1636. HPLC: t_R = 7.5 min, 99.0%

***N*-Cyclohexyl-*N*-((2,2-dimethyl-2*H*-pyrano[2,3-*b*]pyridin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide (20b).** Yield: 60%. ^{1}H NMR ($CDCl_3$): δ 7.89 (s, 1H), 7.49 (s, 1H), 7.44 (d, J = 2.0 Hz, 1H), 7.28 (s, 1H), 6.93 (d, J = 8.8 Hz, 1H), 6.31 (d, J = 10 Hz, 1H), 5.69 (d, J = 10 Hz, 1H), 4.30 (s, 2H), 3.95 (s, 3H), 3.91 (s, 3H), 1.71−1.52 (m, 10 H), 1.29−1.21 ppm (m, 6H). HRMS (ESI) m/z calcd for $C_{25}H_{32}N_2O_5S$ [(M + H)$^+$] 473.2110; found, 473.2127. HPLC: t_R = 11.4 min, 95.0%.

6-Bromo-2,2-dimethyl-2*H*-pyrano[3,2-*b*]pyridine (23). To a solution 2-methyl-3-butyn-2-ol (**21**) in acetonitrile (6 mL) was added

DBU (0.80 mL, 6.61 mmol) at 0 °C. Then TFAA was added dropwise, also at 0 °C. The mixture was stirred for 30 min. In another round-bottom flask, DBU (0.80 mL, 6.61 mmol) was added to a solution of 2-bromo-5-hydroxypyridine (1 g, 5.75 mmol) in 6 mL of acetonitrile at 0 °C. Then 2-methyl-3-butyn-2-ol was added dropwise into this mixture, which was stirred for 30 additional min. The solvent was removed by rotary evaporation, and the residue was diluted with DCM. After separation, the organic layer was washed with 1 M HCl, 1 M NaOH, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuum. The crude product was dissolved in 2 mL of xylene and subjected to microwave irradiation (130 °C, 220 W) for 30 min. The solvent was removed by rotary evaporation and the product concentrated in vacuum. The crude product was purified by column chromatography (silica gel) (10:1 hexane/EtOAc) to give 300 mg of a yellow solid (23% over the two steps). ^{1}H NMR ($CDCl_3$): 1.45 (s, 6H), 5.86 (d, 1H, J = 10.4), 6.44 (d, 1H, J = 10), 6.90 (d, 1H, J = 8.8), 7.14 ppm (s, 1H, J = 8.4). HRMS (ESI) m/z calcd for $C_{10}H_{11}NOBr$ [$(M + H)^+$] 240.0024; found, 240.0026.

2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridine-6-carbaldehyde (24). To a solution of **23** (200 mg, 0.83 mmol) in anhydrous THF (5 mL) at −78 °C was added BuLi (2.5M, 0.35 mL), and the mixture was stirred for 35 min. Then DMF (0.08 mL, 0.1 mmol) was added dropwise. The mixture was stirred at −78 °C for an 30 additional min. Water (3 mL) was added to quench the reaction and was extracted with EtOAc. The organic layer was washed with water, brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash column chromatography, using 20:1 hexane/EtOAc to give the product as a yellowish solid (23% yield). ^{1}H NMR: δ 1.53 (s, 6H), 6.01 (d 1H, J = 10.4), 6.58 (d, 1H, J = 10.4), 7.13 (d, 1H, J = 8.4), 7.77 (d, 1H, J = 8.4), 9.93 ppm (s, 1H). ^{13}C NMR δ 28.8, 78.7, 123.0, 123.3, 123.4, 136.3, 145.8, 153.7, 191.9 ppm

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-aniline (25a).** To a solution of 2,2-dimethyl-2*H*-pyrano[3,2-*b*]-pyridine-6-carbaldehyde (434 mg, 2.28 mmol) in methanol (3 mL) were added aniline (0.3 mL, 2.52 mmol) and zinc chloride (621 mg, 4.56 mmol), and the mixture was stirred at room temperature for 2 h. Then $NaCNBH_3$ (287 mg, 4.56 mmol) was added, and the mixture was stirred overnight. Purification by column chromatography with 4:1 hexane/EtOAc gave an off-white solid. Yield: 48%. ^{1}H NMR ($CDCl_3$): δ 1.49 (s, 6H), 4.361 (s, 2H), 5.91 (d, 1H, J = 10), 6.55 (d, 1H, J = 10), 6.68−6.71 (m, 3H), 7.01 (d, 1H, J = 8.4), 7.08 (d, 1H, J = 8.4), 7.18−7.38 ppm (m, 2H).

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-3,4-dimethoxy-*N*-phenylbenzenesulfonamide (26a).** To a solution of the **25a** (60 mg, 0.237 mmol) in dichloromethane (2.5 mL) were added triethylamine (0.07 mL, 0.474 mmol) and the 3,4-dimethoxybenzenesulfonyl chloride (84 mg, 0.355 mmol). The mixture was stirred for 24 h. The reaction mixture was diluted with DCM and the organic layer washed with water and brine, dried over magnesium sulfate, and concentrated in vacuo. The crude product was purified by column chromatography (silica gel, 3:1 hexane/EtOAc to 1:1 hexane/EtOAc) to give an off-white solid (56 mg). Yield: 50%. Mp 143 °C. ^{1}H NMR ($CDCl_3$): δ 7.29 (dd, J = 8.4, 2.1 Hz, 2H), 7.25−7.17 (m, 3H), 7.14−7.09 (m, 2H), 6.96 (d, J = 8.4 Hz, 1H), 6.92−6.86 (m, 2H), 6.32 (d, J = 10.1 Hz, 1H), 5.80 (d, J = 10.1 Hz, 1H), 4.78 (s, 2H), 3.94 (s, 3H), 3.72 (s, 3H), 1.40 ppm (s, 6H). HRMS (ESI) m/z calcd for $C_{25}H_{26}N_2O_5S$ [$(M + H)^+$] 467.1641; found, 467.1641. HPLC: t_R = 9.7 min, 98.1%.

General Procedure for the Synthesis of 26b−j by Reaction with Alkylsulfonyl Chloride. To a solution of **25** (1 equiv) in methanol was added the primary amine (1 equiv) and $ZnCl_2$ (2 equiv), and the mixture was stirred at room temperature for 2 h. Then $NaCNBH_3$ (2 equiv) was added and the mixture was stirred at room temperature overnight. The solvent was removed by rotary evaporation, and EtOAc was added to the residue. The solid was filtered through Celite and the filtrate washed with 1 M NaOH, water, and brine. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. The crude secondary amine product was used without further purification.

To a solution of the secondary amine (1 equiv) in DCM was added triethylamine (3 equiv) and the sulfonyl chloride (1.5 equiv). The mixture was stirred for 24−48 h. Then water was added and the organic layer extracted with DCM, dried over $MgSO_4$, concentrated under reduced pressure, and purified by flash column chromatography.

***N*-Butyl-*N*-((2,2-dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)-methyl)-3,4-dimethoxybenzenesulfonamide (26b).** Yield: 42%. ^{1}H NMR ($CDCl_3$): δ 7.45 (d, J = 2.4 Hz, 1H), 7.29−7.26 (m, 2H), 7.04 (d, J = 8.4, 1H), 6.94 (d, J = 8.8, 1H), 6.40 (d, J = 8.0, 1H), 5.88 (d, J = 10, 1H), 4.37 (s, 2H), 3.96 (s, 3H), 3.93 (s, 3H), 3.18 (t, J = 7.6, 2H), 1.48 (s, 6H), 1.38 (m, 2H), 1.18 (sx, J = 7.2, 2H), 0.79 ppm (t, J = 7.2, 3H). ^{13}C NMR ($CDCl_3$): δ 152.4, 149.0, 148.8, 148.7, 135.4, 131.6, 123.7, 123.7, 122.7, 121.0, 110.5, 109.8, 56.2, 56.2, 53.3, 48.8, 30.2, 28.2, 19.9, 13.6 ppm. HRMS (ESI) m/z calcd for $C_{23}H_{31}N_2O_5S$ [$(M + H)^+$] 447.1954; observed, 447.1937. HPLC: t_R = 11.4 min, 95.4%.

***N*-(3,4-Dimethoxyphenyl)-*N*-((2,2-dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide (26c).** Yield: 51%. Mp 117 °C. ^{1}H NMR δ ($CDCl_3$): δ 7.32−7.38 (m, 2H), 7.01−6.98 (m, 2H), 6.91 (d, J = 8.4 Hz, 1H), 6.66 (d, J = 8.4 Hz. 1H), 6.68−6.62 (m, 2H), 6.34 (dd, J = 12.0, 0.4 Hz, 1H), 5.82 (d, J = 10.0 Hz, 1H), 4.76 (s, 2H), 3.96 (s, 3H), 3.83 (s, 3H), 3.79 (s, 3H), 3.72 (s, 3H), 1.43 ppm (s, 6H). ^{13}C NMR ($CDCl_3$): δ 152.6, 148.7, 148.7, 148.6, 148.5, 147.8, 140.2, 135.3, 132.2, 129.6, 123.6, 123.6, 122.6, 121.8, 121.0, 112.5, 110.5, 110.3, 56.2, 56.2, 56.1, 55.9, 28.2 ppm. HRMS (ESI) m/z calcd for $C_{27}H_{31}N_2O_7S$ [$(M + H)^+$] 527.1852; found, 527.1866. HPLC: t_R = 7.6 min, 98.4%.

***N*-Cyclopentyl-*N*-((2,2-dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide (26d).** Yield: 31%. ^{1}H NMR ($CDCl_3$): δ 7.55−7.35 (m, 2H), 7.35−7.22 (m, 1H), 7.07 (d, J = 8.4 Hz, 1H), 6.98−6.86 (m, 1H), 6.46 (dd, J = 14.7, 10.2 Hz, 1H), 5.89 (d, J = 10.1 Hz, 1H), 4.44−4.24 (m, 3H), 3.96 (s, 3H), 3.93 (s, 3H), 1.76−1.15 ppm (m, 15H). ^{13}C NMR ($CDCl_3$): δ 152.4, 150.7, 149.0 148.5,140.0 135.4, 132.2, 123.8, 123.7, 121.9, 121.2, 110.5, 109.8, 59.4, 56.3, 56.2, 48.6, 29.1, 28.2, 23.4 ppm. HRMS (ESI) m/z calcd for $C_{24}H_{31}N_2O_5$ [$(M + H)^+$] 459.1954; found, 459.1938. HPLC: t_R = 11.3 min, 96.9%.

***N*-Cyclohexyl-*N*-((2,2-dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide (26e).** Yield: 46%. ^{1}H NMR ($CDCl_3$): 7.47 (dd, J = 2.4, 6.4 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.30 (d, J = 2.4 Hz, 1H), 7.06 (d, J = 8.4, 1H), 6.93 (d, J = 8.8 Hz, 1H), 6.45 (d, J = 10 Hz, 1H), 5.88 (d, J = 10 Hz, 1H), 4.34 (s, 2H), 3.96 (s, 3H), 3.92 (s, 3H), 3.80 (m, 1H), 1.64 (m, 3H), 1.48 (m, 9H), 1.25 −1.20 ppm (m, 4H). ^{13}C NMR ($CDCl_3$): δ 152.2, 150.7, 149.1, 148.5, 140.0, 135.2, 133.2, 123.7, 123.7, 122.5, 120.7, 110.6, 109.5, 58.4, 56.2, 56.1, 48.5, 31.3, 28.2, 26.1, 25.2 ppm. HRMS (ESI) m/z calcd for $C_{25}H_{33}N_2O_5S$ [$(M + H)^+$] 473.2110; found, 473.2118. HPLC: t_R = 13.1 min, 96.8%

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-3,4-dimethoxy-*N*-(5,6,7,8-tetrahydronaphthalen-2-yl)-benzenesulfonamide (26f).** ^{1}H NMR ($CDCl_3$): δ 7.39−7.31 (m, 2H), 6.99 (dd, J = 13.4, 5.2 Hz, 2H), 6.92 (dd, J = 8.1, 2.8 Hz, 2H), 6.81 (d, J = 7.6 Hz, 2H), 6.35 (d, J = 10.1 Hz, 1H), 5.94−5.71 (m, 1H), 4.78 (d, J = 25.5 Hz, 2H), 3.97 (s, 3H), 3.79 (d, J = 5.3 Hz, 3H), 2.65 (dd, J = 25.6, 13.3 Hz, 4H), 1.83−1.66 (m, 4H), 1.41 ppm (d, J = 16.1 Hz, 6H). MS (ESI) [$(M + H)^+$] 521. HPLC: t_R = 15.6 min, 96.9%.

***N*-Cycloheptyl-*N*-((2,2-dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide (26g).** Yield: 18%. Mp 73 °C. ^{1}H NMR ($CDCl_3$): δ 7.47 (dt, J = 4.4, 2.2 Hz, 1H), 7.44 (d, J = 8.4 Hz, 1H), 7.31−7.27 (m, 1H), 7.07 (d, J = 8.4 Hz, 1H), 6.94 (dd, J = 8.5, 4.9 Hz, 1H), 6.45 (dd, J = 10.1, 0.5 Hz, 1H), 5.88 (d, J = 10.1 Hz, 1H), 4.44−4.29 (m, 2H), 3.96 (s, 3H), 3.94 (s, 1H), 3.93 (s, 3H), 1.62−1.29 ppm (m, 18H). HPLC: t_R = 15.2 min, 98.8%.

***N*-Cyclooctyl-*N*-((2,2-dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide (26h).** Yield: 45%. Mp 117 °C. ^{1}H NMR ($CDCl_3$): δ 7.49 (d, J = 6.4, 1H), 7.46 (d, J = 10, 1H), 7.32−7.29 (m, 1H), 7.07 (d, J = 8.0, 1H), 6.94 (d, J = 8.4, 1H), 6.45 (d, J = 10, 1H), 5.89 (d, J = 10, 1H), 4.38 (s, 2H),

3.97−3.93 (m, 7H), 1.61−1.42 ppm (m, 20H). MS (ESI) m/z [(M + H)$^+$] 501. HPLC: t_R = 16.5 min, 99.0%.

***N*-Cyclobutyl-*N*-((2,2-dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide (26i).** Yield: 40%. ^{1}H NMR ($CDCl_3$): δ 7.44 (dd, J = 8.4, 2.2 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 7.31−7.21 (m, 2H), 7.07 (d, J = 8.4 Hz, 1H), 6.94 (t, J = 6.9 Hz, 1H), 6.40 (dd, J = 32.8, 10.0 Hz, 1H), 5.88 (t, J = 10.6 Hz, 1H), 4.49−4.28 (m, 3H), 3.96 (s, 3H), 3.93 (d, J = 3.0 Hz, 3H), 2.08−1.83 (m, 4H), 1.60−1.40 ppm (m, 8H). ^{13}C NMR ($CDCl_3$): δ 152.5, 150.3, 149.0, 148.6, 140.1, 135.4, 131.7, 123.8, 123.7, 121.8, 121.0, 110.5, 109.6, 77.4, 77.0, 76.7, 56.2, 56.2, 52.7, 49.3, 28.9, 28.2, 15.0 ppm. MS (ESI+) m/z [(M + H)$^+$] 445. HPLC: t_R = 8.7 min, 97%.

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-*N*-(4-fluorophenyl)-3,4-dimethoxybenzenesulfonamide (26j).** Yield: 12%. ^{1}H NMR ($CDCl_3$): δ 7.55−7.40 (m, 1H), 7.38−7.23 (m, 3H), 7.16−7.01 (m, 4H), 7.00−6.87 (m, 5H), 6.40 (t, J = 11.7 Hz, 1H), 5.88 (t, J = 13.4 Hz, 1H), 4.83 (d, J = 14.0 Hz, 2H), 3.97 (s, 3H), 3.86−3.73 (m, 3H), 1.44 ppm (s, 6H). HRMS (ESI) m/z calcd for $C_{25}H_{25}FN_2O_5S$ [(M + H)$^+$] 485.1546; found, 485.1531. HPLC: t_R = 10.2 min, 97.6%.

General Procedure for 26k−t. To a solution of **25a** (1 equiv) in pyridine at 0 °C was added the appropriate sulfonyl chloride dropwise. The mixture was allowed to warm to room temperature overnight. The reaction mixture was then diluted with EtOAc, and the organic layer was washed with 10% citric acid, saturated $NaHCO_3$, water, and brine. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The crude product was purified by flash column chromatography (silica gel).

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-*N*-phenylcyclohexanesulfonamide (26k).** Yield: 60%. ^{1}H NMR ($CDCl_3$): δ 7.30−7.22 (m, 4H), 7.07−7.01 (m, 2H), 6.90 (dd, J = 24.2, 1.8 Hz, 1H), 6.46 (dd, J = 29.3, 2.1 Hz, 1H), 5.86 (d, J = 29.3 Hz, 1H), 5.05 (d, J = 48.2 Hz, 1H), 4.90 (d, J = 48.2 Hz, 1H), 2.13−1.96 (m, 4H), 1.78−1.70 (m, 5H), 1.44 (d, J = 6.3 Hz, 6H), 1.27 ppm (m, 2H). ^{13}C NMR ($CDCl_3$): δ 148.5, 148.3, 143.6, 140.4, 135.2, 129.1, 123.9, 123.8, 123.6, 122.0, 121.8, 92.4, 34.5, 31.8, 28.3, 28.2, 24.7, 21.7, 21.3 ppm. MS (ESI+) m/z [(M + Na)$^+$] 435. HPLC: t_R = 10.16 min, 97.6%.

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-*N*-phenylpropane-2-sulfonamide (26l).** Yield: 58%. ^{1}H NMR ($CDCl_3$): δ 7.37−7.19 (m, 4H), 7.16−6.99 (m, 2H), 6.93 (t, J = 9.4 Hz, 1H), 6.46 (dd, J = 10.1, 0.5 Hz, 1H), 5.87 (d, J = 10.1 Hz, 1H), 4.92 (dd, J = 36.5, 16.5 Hz, 2H), 1.81 (s, 3H), 1.75 (d, J = 11.8 Hz, 4H), 1.45 ppm (t, J = 5.8 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 148.5, 148.2, 143.1, 140.5, 135.3, 129.1, 124.2, 123.7, 123.6, 122.2, 122.1, 86.0, 28.3, 28.2, 27.4, 25.4 ppm. MS (ESI+) m/z [(M + H)$^+$] 373. HPLC: t_R = 10.0 min, 97.8%.

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-*N*-phenylcyclopropanesulfonamide (26m).** Yield: 46%. ^{1}H NMR ($CDCl_3$): δ 7.44−7.42 (m, 2H), 7.34−7.23 (m, 4H), 6.99 (d, J = 8 Hz, 1H), 6.40 (d, J = 10, 1H), 5.85 (dd, J = 10, 1H), 4.98 (s, 2H), 2.55 (m, 1H), 1.44 (d, J = 16.1 Hz, 6H), 1.13−1.11 (m, 2H), 0.98−0.95 ppm (m, 2H). ^{13}C NMR ($CDCl_3$): δ 148.7, 148.1, 140.4, 139.7, 135.3, 129.1, 128.8, 127.7, 123.7, 123.6, 122.4, 56.2, 28.6, 28.2, 5.16 ppm. HPLC: t_R = 8.31 min, 95.8%. HRMS (ESI) m/z calcd for $C_{20}H_{22}N_2O_3S$ [(M + Na)$^+$] 393.1212; found, 393.1231. HPLC: t_R = 10.1 min, 98.3%.

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-*N*-phenylbutane-1-sulfonamide (26n).** Yield: 48%. ^{1}H NMR ($CDCl_3$): δ 7.41−7.29 (m, 4H), 7.27−7.22 (m, 1H), 7.18 (d, J = 8.3 Hz, 1H), 7.00 (dd, J = 12.4, 5.5 Hz, 1H), 6.41 (d, J = 10.1 Hz, 1H), 5.86 (d, J = 10.1 Hz, 1H), 4.94 (s, 2H), 3.38−2.79 (m, 2H), 1.96−1.77 (m, 2H), 1.54−1.38 (m, 8H), 1.12−0.77 ppm (m, 3H). ^{13}C NMR ($CDCl_3$): δ 170.85, 148.78, 148.19, 139.60, 135.37, 129.28, 128.34, 127.62, 123.68, 123.62, 122.54, 56.25, 51.28, 28.22, 25.29, 21.68, 13.61 ppm. MS (ESI) m/z [(M + H)$^+$] 387. HPLC: t_R = 8.3 min, 95.8%.

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-*N*-phenylpropane-1-sulfonamide (26o).** Yield: 20%. Mp 120 °C. ^{1}H NMR ($CDCl_3$): δ 7.40−7.21 (m, 6H), 7.17 (d, J = 8.3 Hz, 1H), 6.98 (t, J = 7.2 Hz, 1H), 6.40 (d, J = 10.1 Hz, 1H), 5.85 (d, J = 10.1 Hz, 1H), 4.93 (s, 2H), 3.20−2.99 (m, 2H), 1.98−1.79 (m, 2H), 1.43 (s, 6H), 1.03 ppm (t, J = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 148.78, 148.18, 139.59, 135.38, 129.28, 128.35, 127.62, 123.67, 123.63, 122.53, 77.34, 77.02, 76.70, 56.19, 53.17, 28.21, 17.08, 13.09 ppm. MS (ESI) m/z [(M + H)$^+$] 373. HPLC: t_R = 9.23 min, 97.4%.

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-2-methyl-*N*-phenylpropane-1-sulfonamide (26p).** Yield: 28%. ^{1}H NMR ($CDCl_3$): δ 7.40−7.30 (m, 4H), 7.27−7.21 (m, 1H), 7.19 (d, J = 8.3 Hz, 1H), 6.99 (d, J = 8.3 Hz, 1H), 6.42 (d, J = 10.1 Hz, 1H), 5.89 (dd, J = 21.3, 10.0 Hz, 1H), 4.93 (s, 2H), 3.01 (dd, J = 14.1, 6.5 Hz, 2H), 2.34 (dd J = 13.3, 6.7 Hz, 1H), 1.47 (d, J = 14.5 Hz, 6H), 1.10 ppm (d, J = 6.7 Hz, 6H). MS (ESI) m/z [(M + H)$^+$] 387. HPLC: t_R = 10.4 min, 96.3%.

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-*N*-phenylbiphenyl-4-sulfonamide (26q).** Yield: 17%. Mp 170 °C. ^{1}H NMR ($CDCl_3$): δ 7.69 (s, 4H), 7.67−7.60 (m, 2H), 7.57−7.41 (m, 3H), 7.36−7.22 (m, 5H), 7.21−7.13 (m, 2H), 7.00 (t, J = 7.3 Hz, 1H), 6.34 (d, J = 10.1 Hz, 1H), 5.83 (t, J = 9.5 Hz, 1H), 4.86 (d, J = 5.9 Hz, 2H), 1.43 ppm (s, 6H). HRMS (ESI) m/z calcd for $C_{29}H_{27}N_2O_3S$ [(M + H)$^+$] 483.1742; found, 483.1723. HPLC: t_R = 16.6 min, 96.1%.

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-*N*-phenylbenzo[*d*][1,3]dioxole-4-sulfonamide (26r).** Yield: 65%. Mp 187 °C. ^{1}H NMR ($CDCl_3$): δ 7.35−7.21 (m, 4H), 7.21−7.12 (m, 3H), 7.08 (t, J = 3.9 Hz, 1H), 7.02−6.93 (m, 1H), 6.88−6.78 (m, 1H), 6.36 (dd, J = 10.1, 0.5 Hz, 1H), 6.09 (s, 2H), 5.84 (dd, J = 14.0, 9.0 Hz, 1H), 4.82 (s, 2H), 1.68 (s, 2H), 1.43 ppm (s, 6H). HRMS (ESI) m/z calcd for $C_{24}H_{23}N_2O_5S$ [(M + H)$^+$] 451.1328; found, 451.1316. HPLC: t_R = 10.7 min, 99.7%.

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-*N*-phenylquinoline-8-sulfonamide (26s).** Yield: 65%; mp 149 °C. ^{1}H NMR ($CDCl_3$): δ 9.19 (dd, J = 4.2, 1.8 Hz, 1H), 8.43−8.16 (m, 2H), 8.00 (dt, J = 10.5, 5.3 Hz, 1H), 7.66−7.56 (m, 2H), 7.54−7.47 (m, 1H), 7.14−6.94 (m, 6H), 6.40 (d, J = 10.1 Hz, 1H), 5.85 (t, J = 15.1 Hz, 1H), 5.58 (d, J = 35.1 Hz, 2H), 1.45 ppm (s, 6H). ^{13}C NMR ($CDCl_3$): δ 151.3, 150,0, 148.5, 144.2, 140.1, 139.6, 137.1, 136.5, 135.1, 133.7, 133.5, 128.8, 128.8, 128.2, 127.2, 125.4, 123.9, 123.8, 122.4, 122.1, 77.4, 77.1, 76.9, 76.7, 58.8, 28.2 ppm. HRMS (ESI) m/z calcd for $C_{26}H_{24}N_3O_3S$ [(M + H)$^+$] 485.1538; found, 485.1543. HPLC: t_R = 10.0 min, 96.9%.

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-*N*-phenyl-2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamide (26t).** Yield: 57%. Mp 131 °C. ^{1}H NMR ($CDCl_3$): δ 1.419 (s, 6H), 4.33−4.28 (m, 2H), 4.81 (s, 2H), 5.82 (d, 1H, J = 10), 6.35 (d, 1H, J = 10), 6.89 (d, 1H, J = 8.4), 6.97 (d, 1H, J = 8.4), 7.06 (dd, 1H), 7.12−7.15 (m, 2H), 7.20−7.30 ppm (m, 5H). ^{13}C NMR ($CDCl_3$): δ 148.6, 147.7, 147.5, 143.4, 140.2, 139.4, 135.2, 130.4, 128.8, 128.5, 127.6, 123.7, 123.6, 122.4, 121.6, 117.4, 117.4, 77.4, 77.1, 77.0, 76.7, 64.6, 64.1, 60.4, 55.7, 28.2, 21.1, 14.2 ppm. HRMS (ESI) m/z calcd for $C_{15}H_{15}N_2O_5S$ [(M + H)$^+$] 465.1484; found, 465.1489. HPLC: t_R = 10.9 min, 98.2%.

***N*-((2,2-Dimethyl-2*H*-pyrano[3,2-*b*]pyridin-6-yl)methyl)-3,4-dimethoxy-*N*-phenylbenzamide (27).** To a solution of **25a** (60 mg, 0.226 mmol) in DCM (3 mL) were added triethylamine (0.06 mL, 0.452 mmol) and 3,4-dimethoxybenzoyl chloride (54 mg, 0.271 mmol). The mixture was stirred overnight at room temperature. The reaction mixture was washed with H_2O (×2) and saturated $NaHCO_3$ (×2), dried over $MgSO_4$, and concentrated in vacuo. Purification by column chromatography with silica gel and 3:1 DCM/EtOAc gave a quantitative yield of a light yellow oil. ^{1}H NMR ($CDCl_3$): δ 7.26−7.17 (m, 3H), 7.17−7.08 (m, 3H), 7.06−6.97 (m, 2H), 6.95 (t, J = 5.0 Hz, 1H), 6.66 (d, J = 8.4 Hz, 1H), 6.53−6.40 (m, 1H), 5.92−5.79 (m, 1H), 5.16 (s, 2H), 3.83 (s, 3H), 3.66 (s, 3H), 1.46 ppm (s, 6H). ^{13}C NMR ($CDCl_3$): δ 169.9, 150.3, 149.0, 148.6, 147.9, 144.7, 140.6, 135.1, 129.0, 127.8, 127.2, 126.3, 124.0, 123.6, 123.0, 122.3, 112.6, 109.9, 77.4, 77.0, 77.0, 76.7, 55.8, 55.7, 55.6, 28.2 ppm. HRMS (ESI) m/z calcd for $C_{26}H_{27}N_2O_4$ [(M + H)$^+$] 431.1971; found, 431.1951.

2-Bromo-6-(hydroxymethyl)pyridin-3-ol (30). A solution of 2-bromo-3-hydroxy-6-methylpyridine 1-oxide **29** (15 g, 0.075 mol) in TFAA (50 mL, 0.375 mol) was stirred at 40 °C for 24 h. The solvent was removed under vacuum. The residue was purified by column chromatography (silica gel, EtOAc/hexane, 2:1). Yield: 4.5 g, 30%.

^{1}H NMR ($CDCl_3$): δ 7.32 (d, J = 8.0 Hz, 1H), 7.25 (d, J = 8.5 Hz, 1H), 4.56 (s, 2H).

(6-Bromo-5-(2-methylbut-3-yn-2-yloxy)pyridin-2-yl)-methanol (31). Compound **30** (1.02 g, 5 mmol) was dissolved in acetone (20 mL. Then K_2CO_3 (166 mg, 7 mmol), KI (33.2 mg, 0.2 mmol), and $CuCl_2.2H_2O$ (33.2 mg, 0.02 mmol) were added. The suspension was stirred at 60 °C for 10 min. The solution of 3-chloro-3-methyl-2-butyne (1.02 g, 5 mmol) in acetone (5 mL) was added dropwise to the solution of **30**. The reaction mixture was cooled to room temperature and the suspension filtered. The solid residue was washed with MeOH. The filtrate was concentrated under vacuum and purified with column chromatography (silica gel, EtOAc/hexane, 1:1). Yield: 700 mg, 57%. ^{1}H NMR ($CDCl_3$): δ 7.88 (d, J = 8.0 Hz, 1H), 7.23 (d, J = 8.0 Hz, 1H), 4.72 (s, 2H), 1.73 ppm (s, 6H). ^{13}C NMR ($CDCl_3$): δ 154.7, 149.6, 137.03, 129.5, 120.4, 85.7, 75.8, 75.7, 64.6, 30.1 ppm.

(8-Bromo-2,2-dimethyl-2*H*-pyrano[2,3-*c*]pyridin-6-yl)-methanol (32). A solution of **31** (700 mg, 2.5 mmol) in toluene (10 mL) was subjected to microwave irradiation (200 W, 120 °C) for 1 h. The reaction mixture was cooled to room temperature and filtered. The filtrate was concentrated under vacuum and the residue purified by column chromatography (silica gel, EtOAc/hexane, 1:3 to 1:2). Yield: 500 mg, 70%. ^{1}H NMR ($CDCl_3$): δ 6.88 (s, 1H), 6.28 (d, J = 10 Hz, 1H), 5.90 (d, J = 9.6 Hz, 1H), 4.63 (s, 2H), 1.51 ppm (s, 6H).

8-Bromo-6-(bromomethyl)-2,2-dimethyl-2*H*-pyrano[2,3-*c*]-pyridine (33). To a solution of **32** in DCM (2 mL) was added CBr_4 (66 mg, 0.2 mmol) and PPh_3 (264 mg, 0.2 mmol). The reaction mixture was stirred at room temperature for 1 h. The solvent was removed under vacuum and the residue purified by column chromatography (silica gel, EtOAc/hexane, 1:4). Yield: 260 mg, 40%. ^{1}H NMR ($CDCl_3$): δ 6.28 (d, J = 10 Hz, 1H), 5.91 (d, J = 10 Hz, 1H), 4.47 (s, 2H), 1.53 ppm (s, 6H).

General Procedure for the Synthesis of 34. To a degassed flask with **33** (1 equiv) were added DMF, aniline (1.5 equiv), and DIEA (1.5 equiv). The mixture was stirred at room temperature overnight. Water (50 mL) was added to the reaction mixture, and the resulting solution was extracted with ethyl acetate (3 × 25 mL). The combined organic layers were washed with 0.5 N HCl (50 mL), 40% $NaHCO_3$ (50 mL), water (50 mL), and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by column chromatography (silica gel).

***N*-((8-Bromo-2,2-dimethyl-2*H*-pyrano[2,3-*c*]pyridin-6-yl)-methyl)benzenamine 34a.** Yield: 78%. ^{1}H NMR ($CDCl_3$): δ 7.26−7.21 (m, 3H), 6.97−6.95 (m, 3H), 6.23 (d, J = 9.6 Hz, 1H), 5.85 (d, J = 9.6 Hz, 1H), 4.40 (s, 2H), 1.48 ppm (s, 6 H).

***N*-((8-Bromo-2,2-dimethyl-2*H*-pyrano[2,3-*c*]pyridin-6-yl)-methyl)cyclohexanamine (34b).** Yield: 60%. ^{13}C NMR (CD_3OD): δ 152.0, 145.2, 136.9, 129.7, 129.1, 119.8, 118.7, 78.5, 56.0, 49.8, 32.4, 26.8, 25.8, 24.7 ppm.

General Procedure for the Synthesis of Compound 35. A flask of secondary amine **34** (1 equiv) was degassed, and THF (anhydrous) was added under nitrogen. The solution was cooled to −78 °C and stirred for 1 h. BuLi (2.5 equiv) was the added to the solution dropwise at −78 °C. The resulting solution was stirred for 1 h. Water (10 mL) was added to the solution, which was diluted with ethyl acetate (25 mL). After separation, the aqueous layer was extracted with ethyl acetate and washed with water (25 mL × 3) and brine (25 mL), dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by column chromatography (silica gel, EtOAc/hexane, 1:4)

***N*-((2,2-Dimethyl-2*H*-pyrano[2,3-*c*]pyridin-6-yl)methyl)-benzenamine (35a).** Yield: 70%. ^{1}H NMR (CD_3OD): δ 7.91 (s, 1H), 7.08−7.04 (m, 3H), 6.59−6.57 (m, 3H), 6.28 (d, J = 9.6 Hz, 1H), 5.92 (s, J = 10 Hz, 1H), 4.88 (s, 2H), 1.41 ppm (s, 6H). ^{13}C NMR (CD_3OD): δ 152.3, 148.3, 148.2, 136.7, 135.2, 128.9, 128.7, 119.9, 117.8, 116.8, 112.6, 76.9, 26.8 ppm.

***N*-((2,2-Dimethyl-2*H*-pyrano[2,3-*c*]pyridin-6-yl)methyl)-cyclohexanamine (35b).** Yield: 50%. ^{1}H NMR (CD_3OD): δ 7.95 (s, 1H), 7.08 (s, 1H), 6.44 (d, J = 9.6 Hz, 1H), 6.03 (d, J = 10 Hz, 1H), 3.78 (s, 2H), 2.46 (m, 1H), 1.97−1.75 (m, 5 H), 1.46 (s, 6H, 1.27−1.16 ppm (m, 5H). ^{13}C NMR (CD_3OD): δ 151.5, 148.3, 136.7, 136.5, 128.7, 119.9, 119.0, 77.00, 56.0, 50.3, 32.4, 26.8, 25.8, 24.7 ppm.

General Procedure for Synthesis of 36. A mixture of compound **35** (1 equiv) and the appropriate sulfonyl chloride (2 equiv) in pyridine was stirred overnight at room temperature. Then 1 M HCl was added to the reaction mixture and the solution extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with water (3 × 20 mL), brine and dried over Na_2SO_4. The solvent was removed under vacuum and the residue purified by column chromatography (silica gel, EtOAc/hexane, 1:4).

***N*-((2,2-Dimethyl-2*H*-pyrano[2,3-*c*]pyridin-6-yl)methyl)-4-methoxy-*N*-phenylbenzenesulfonamide (36a).** Yield: 50%. ^{1}H NMR (CD_3OD): δ 7.76 (s, 1H), 7.59−7.56 (m, 2H), 7.25−7.18 (m, 3H), 7.09−7.04 (m, 4H), 6.37 (d, J = 10 Hz, 1H), 5.97 (d, J = 10 Hz, 1H), 4.88 (s, 3H), 4.78 (s, 2H), 3.084 (s, 3H), 1.40 ppm (s, 6H). ^{13}C NMR (CD_3OD): 163.5, 148.6, 139.3, 136.7, 136.1, 129.7, 129.3, 128.8, 128.5, 128.5, 127.6, 119.7, 119.4, 113.9, 77.1, 55.0, 54.9, 26.8 ppm. MS (ESI) m/z [(M + H)$^+$] 437. HPLC: t_R = 9.6 min, 97.8%.

***N*-((2,2-Dimethyl-2*H*-pyrano[2,3-*c*]pyridin-6-yl)methyl)-4-nitro-*N*-phenylbenzenesulfonamide (36b).** Yield: 59%. ^{1}H NMR (CD_3OD): δ 8.41 (dd, J = 2.0 Hz, 4.8 Hz, 2H), 7.89 (dd, J = 2.0 Hz, 4.8 Hz), 7.78 (s, 1H), 7.29−7.28 (m, 4H), 7.10−7.08 (m, 2H), 6.39 (d, J = 10 Hz, 1H), 6.00 (d, J = 10 Hz, 1H), 1.42 ppm (s, 6H). MS (ESI) m/z [(M + H)$^+$] 452. HPLC: t_R = 9.03 min, 95.2%.

***N*-Cyclohexyl-*N*-((2,2-dimethyl-2*H*-pyrano[2,3-*c*]pyridin-6-yl)methyl)-4-isopropylbenzenesulfonamide (36c).** Yield: 28%. ^{1}H NMR ($CDCl_3$): δ 7.98 (s, 1H), 7.77−7.75 (m, 2H), 7.37−7.34 (m, 1H), 7.28 (d, J = 8.8 Hz, 1H), 6.34 (d, J = 9.6 Hz, 1H), 5.84 (d, J = 10 Hz, 1H), 4.42 (s, 2H), 3.80 (m, 1H), 2.99 (m, 1H), 1.75−1.59 (m, 3H), 1.55−1.39 (m, 9 H), 1.29−1.27 (m, 6H), 1.23 −1.19 ppm (m, 4H). ^{13}C NMR ($CDCl_3$): δ 153.8, 151.8, 148.1, 138.7, 136.7, 135.8, 128.1, 127.1, 127.3, 120.9, 118.9, 58.5, 48.7, 34.1, 28.0, 26.1, 25.1, 23.7 ppm. MS (ESI) m/z [(M + H)$^+$] 455. HPLC: t_R = 18.6 min, 98.2%.

***N*-Cyclohexyl-*N*-((2,2-dimethyl-2*H*-pyrano[2,3-*c*]pyridin-6-yl)methyl)-3,4-dimethoxybenzenesulfonamide (36d).** Yield: 28%. ^{1}H NMR ($CDCl_3$) δ 8.00 (s, 1H), 7.50−7.47 (m, 1H), 7.33−7.29 (m, 2H), 6.94 (d, J = 8.4 Hz, 1H), 6.36 (d, J = 9.6 Hz, 1H), 5.86 (d, J = 9.6 Hz, 1H), 5.32 (s, 2H), 3.967 (s, 3H), 3.94 (s, 3H), 3.97 (m, 1H), 1.68 −1.52 (m, 4H), 1,48 (s, 6H), 1.28−1.20 ppm (m, 4H). ^{13}C NMR ($CDCl_3$): δ 151.7, 149.1, 136.7, 135.9, 133.2, 128.1, 120.8, 120.7, 118.9, 110.6, 109.5, 83.1, 58.5, 56.2, 56.2, 48.6, 31.4, 28.0, 26.1, 25.1 ppm. MS (ESI) m/z [(M + H)$^+$] 473. HPLC: t_R = 10.3 min, 97.3%.

AUTHOR INFORMATION

Corresponding Author

*For E.G.V.M.: telephone, 1-404-778-5563; e-mail, evanmei@emory.edu. For B.W.: telephone, 1-404-413-5544; e-mail, wang@gsu.edu.

ACKNOWLEDGMENTS

Financial support from the National Institutes of Health (Grant R01CA116804 to E.V.G.M.), V Foundation (to E.V.G.M), and an associated minority supplement (Grant R01CA116804-S1) and a fellowship from the Georgia State University Molecular Basis of Diseases Program (to S.R.M.) is gratefully acknowledged. The authors thank Sarah Burroughs for her help with the submission of this manuscript

ABBREVIATIONS USED

AP, alkaline phosphatase; CBP, CREB-binding protein; HIF, hypoxia inducible factor; HRE, hypoxia-responsive element; ODDD, oxygen-dependent degradation domain; PHD, prolyl hydroxylase; VEGF, vascular endothelial growth factor; pVHL, von Hippel−Lindau protein

REFERENCES

(1) Denko, N. C. Hypoxia, HIF1 and glucose metabolism in the solid tumour. *Nat. Rev. Cancer* **2008**, *8* (9), 705−713.

(2) Pouyssegur, J.; Dayan, F.; Mazure, N. M. Hypoxia signalling in cancer and approaches to enforce tumour regression. *Nature* **2006**, *441* (7092), 437−443.

(3) Kaur, B.; Tan, C.; Brat, D. J.; Post, D. E.; Van Meir, E. G. Genetic and hypoxic regulation of angiogenesis in gliomas. *J. Neuro-Oncol.* **2004**, *70* (2), 229−243.

(4) Brown, J. M.; Giaccia, A. J. The unique physiology of solid tumors: opportunities (and problems) for cancer therapy. *Cancer Res.* **1998**, *58* (7), 1408−1416.

(5) Harris, A. L. Hypoxia: a key regulatory factor in tumour growth. *Nat. Rev. Cancer* **2002**, *2* (1), 38−47.

(6) Greijer, A. E.; van der Groep, P.; Kemming, D.; Shvarts, A.; Semenza, G. L.; Meijer, G. A.; van de Wiel, M. A.; Belien, J. A.; van Diest, P. J.; van der Wall, E. Up-regulation of gene expression by hypoxia is mediated predominantly by hypoxia-inducible factor 1 (HIF-1). *J. Pathol.* **2005**, *206* (3), 291−304.

(7) Semenza, G. L. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* **2010**, *29* (5), 625−634.

(8) Kaelin, W. G. Jr.; Ratcliffe, P. J. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol. Cell* **2008**, *30* (4), 393−402.

(9) Kaluz, S.; Kaluzova, M.; Stanbridge, E. J. Does inhibition of degradation of hypoxia-inducible factor (HIF) alpha always lead to activation of HIF? Lessons learnt from the effect of proteasomal inhibition on HIF activity. *J. Cell. Biochem.* **2008**, *104* (2), 536−544.

(10) Ohh, M.; Park, C. W.; Ivan, M.; Hoffman, M. A.; Kim, T. Y.; Huang, L. E.; Pavletich, N.; Chau, V.; Kaelin, W. G. Ubiquitination of hypoxia-inducible factor requires direct binding to the beta-domain of the von Hippel−Lindau protein. *Nat. Cell Biol.* **2000**, *2* (7), 423−427.

(11) Bruick, R. K.; McKnight, S. L. A conserved family of prolyl-4-hydroxylases that modify HIF. *Science* **2001**, *294* (5545), 1337−1340.

(12) Epstein, A. C. R.; Gleadle, J. M.; McNeill, L. A.; Hewitson, K. S.; O'Rourke, J.; Mole, D. R.; Mukherji, M.; Metzen, E.; Wilson, M. I.; Dhanda, A.; Tian, Y. M.; Masson, N.; Hamilton, D. L.; Jaakkola, P.; Barstead, R.; Hodgkin, J.; Maxwell, P. H.; Pugh, C. W.; Schofield, C. J.; Ratcliffe, P. J. *C. elegans* EGL-9 and mammalian homologs define a family of dioxygenases that regulate HIF by prolyl hydroxylation. *Cell* **2001**, *107* (1), 43−54.

(13) Maxwell, P. H. The HIF pathway in cancer. *Semin. Cell Dev. Biol.* **2005**, *16* (4−5), 523−530.

(14) Schofield, C. J.; Ratcliffe, P. J. Oxygen sensing by HIF hydroxylases. *Nat. Rev. Mol. Cell Biol.* **2004**, *5* (5), 343−354.

(15) Kaur, B.; Khwaja, F. W.; Severson, E. A.; Matheny, S. L.; Brat, D. J.; Van Meir, E. G. Hypoxia and the hypoxia-inducible-factor pathway in glioma growth and angiogenesis. *Neuro-Oncol.* **2005**, *7* (2), 134−153.

(16) Tsuzuki, Y.; Fukumura, D.; Oosthuyse, B.; Koike, C.; Carmeliet, P.; Jain, R. K. Vascular endothelial growth factor (VEGF) modulation by targeting hypoxia-inducible factor-1 alpha → hypoxia response element → VEGF cascade differentially regulates vascular response and growth rate in tumors. *Cancer Res.* **2000**, *60* (22), 6248−6252.

(17) Bos, R.; van der Groep, P.; Greijer, A. E.; Shvarts, A.; Meijer, S.; Pinedo, H. M.; Semenza, G. L.; van Diest, P. J.; van der Wall, E. Levels of hypoxia-inducible factor-1alpha independently predict prognosis in patients with lymph node negative breast carcinoma. *Cancer* **2003**, *97* (6), 1573−1581.

(18) Talks, K. L.; Turley, H.; Gatter, K. C.; Maxwell, P. H.; Pugh, C. W.; Ratcliffe, P. J.; Harris, A. L. The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. *Am. J. Pathol.* **2000**, *157* (2), 411−421.

(19) Zhong, H.; De Marzo, A. M.; Laughner, E.; Lim, M.; Hilton, D. A.; Zagzag, D.; Buechler, P.; Isaacs, W. B.; Semenza, G. L.; Simons, J. W. Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. *Cancer Res.* **1999**, *59* (22), 5830−5835.

(20) Aebersold, D. M.; Burri, P.; Beer, K. T.; Laissue, J.; Djonov, V.; Greiner, R. H.; Semenza, G. L. Expression of hypoxia-inducible factor-1alpha: a novel predictive and prognostic parameter in the radiotherapy of oropharyngeal cancer. *Cancer Res.* **2001**, *61* (7), 2911−2916.

(21) Birner, P.; Gatterbauer, B.; Oberhuber, G.; Schindl, M.; Rossler, K.; Prodinger, A.; Budka, H.; Hainfellner, J. A. Expression of hypoxia-inducible factor-1 alpha in oligodendrogliomas: its impact on prognosis and on neoangiogenesis. *Cancer* **2001**, *92* (1), 165−171.

(22) Birner, P.; Schindl, M.; Obermair, A.; Plank, C.; Breitenecker, G.; Oberhuber, G. Overexpression of hypoxia-inducible factor 1alpha is a marker for an unfavorable prognosis in early-stage invasive cervical cancer. *Cancer Res.* **2000**, *60* (17), 4693−4696.

(23) Koukourakis, M. I.; Bentzen, S. M.; Giatromanolaki, A.; Wilson, G. D.; Daley, F. M.; Saunders, M. I.; Dische, S.; Sivridis, E.; Harris, A. L. Endogenous markers of two separate hypoxia response pathways (hypoxia inducible factor 2 alpha and carbonic anhydrase 9) are associated with radiotherapy failure in head and neck cancer patients recruited in the CHART randomized trial. *J. Clin. Oncol.* **2006**, *24* (5), 727−735.

(24) Post, D. E.; Devi, N. S.; Li, Z.; Brat, D. J.; Kaur, B.; Nicholson, A.; Olson, J. J.; Zhang, Z.; Van Meir, E. G. Cancer therapy with a replicating oncolytic adenovirus targeting the hypoxic microenvironment of tumors. *Clin. Cancer Res.* **2004**, *10* (24), 8603−8612.

(25) Post, D. E.; Sandberg, E. M.; Kyle, M. M.; Devi, N. S.; Brat, D. J.; Xu, Z.; Tighiouart, M.; Van Meir, E. G. Targeted cancer gene therapy using a hypoxia inducible factor dependent oncolytic adenovirus armed with interleukin-4. *Cancer Res.* **2007**, *67* (14), 6872−6881.

(26) Post, D. E.; Van Meir, E. G. Generation of bidirectional hypoxia/HIF-responsive expression vectors to target gene expression to hypoxic cells. *Gene Ther.* **2001**, *8* (23), 1801−1807.

(27) Post, D. E.; Van Meir, E. G. A novel hypoxia-inducible factor (HIF) activated oncolytic adenovirus for cancer therapy. *Oncogene* **2003**, *22* (14), 2065−2072.

(28) Melillo, G. Inhibiting hypoxia-inducible factor 1 for cancer therapy. *Mol. Cancer Res.* **2006**, *4* (9), 601−605.

(29) Semenza, G. L. Evaluation of HIF-1 inhibitors as anticancer agents. *Drug Discovery Today* **2007**, *12* (19−20), 853−859.

(30) Belozerov, V. E.; Van Meir, E. G. Hypoxia inducible factor-1: a novel target for cancer therapy. *Anticancer Drugs* **2005**, *16* (9), 901−909.

(31) Fujii, M.; Egawa, K.; Hirai, Y.; Kondo, M.; Akita, H.; Nose, K.; Toriizuka, K.; Ida, Y. First synthesis of racemic methylophiopogonanone B and its inhibitory activity of hypoxia-inducible factor-1 alpha. *Heterocycles* **2009**, *78* (1), 207−212.

(32) Nicolaou, K. C.; Pfefferkorn, J. A.; Mitchell, H. J.; Roecker, A. J.; Barluenga, S.; Cao, G. Q.; Affleck, R. L.; Lillig, J. E. Natural product-like combinatorial libraries based on privileged structures. 2. Construction of a 10 000-membered benzopyran library by directed split-and-pool chemistry using NanoKans and optical encoding. *J. Am. Chem. Soc.* **2000**, *122* (41), 9954−9967.

(33) Tan, C.; de Noronha, R. G.; Roecker, A. J.; Pyrzynska, B.; Khwaja, F.; Zhang, Z.; Zhang, H.; Teng, Q.; Nicholson, A. C.; Giannakakou, P.; Zhou, W.; Olson, J. J.; Pereira, M. M.; Nicolaou, K. C.; Van Meir, E. G. Identification of a novel small-molecule inhibitor of the hypoxia-inducible factor 1 pathway. *Cancer Res.* **2005**, *65* (2), 605−612.

(34) Narita, T.; Yin, S.; Gelin, C. F.; Moreno, C. S.; Yepes, M.; Nicolaou, K. C.; Van Meir, E. G. Identification of a novel small molecule HIF-1alpha translation inhibitor. *Clin. Cancer Res.* **2009**, *15* (19), 6128−6136.

(35) Tan, C.; de Noronha, R. G.; Devi, N. S.; Jabbar, A. A.; Kaluz, S.; Liu, Y.; Mooring, S. R.; Nicolaou, K. C.; Wang, B.; Van Meir, E. G. Sulfonamides as a new scaffold for hypoxia inducible factor pathway inhibitors. *Bioorg. Med. Chem. Lett.* **2011**, *21* (18), 5528−5532.

(36) Van Meir, E. G.; et al. A novel class of anti-tumor agents that modulate the p300/CBP HIF-1 interaction and inhibit hypoxia signaling. Manuscript in preparation.

(37) Grossniklaus, H. E.; et al. Efficacy of the novel small molecule HIF pathway inhibitor KCN1 in the control of micrometastases in experimental ocular melanoma. Manuscript in preparation.
(38) Liebmann, J.; Cook, J. A.; Mitchell, J. B. Cremophor EL, solvent for paclitaxel, and toxicity. *Lancet* **1993**, *342* (8884), 1428.
(39) Prado, S.; Janin, Y. L.; Saint-Joanis, B.; Brodin, P.; Michel, S.; Koch, M.; Cole, S. T.; Tillequin, F.; Bost, P.-E. Synthesis and antimycobacterial evaluation of benzofurobenzopyran analogues. *Bioorg. Med. Chem.* **2007**, *15* (5), 2177−2186.
(40) Evans, J. M.; Stemp, G. Short efficient syntheses of 2,2-dimethyl-2*H*-pyranopyridines. *Synth. Commun.* **1988**, *18* (10), 1111−1118.
(41) Robert, N.; Hoarau, C.; Celanire, S.; Ribereau, P.; Godard, A.; Queguiner, G.; Marsais, F. Efficient and fast Heck vinylation of 2-bromo-6-methyl pyridines with methylacrylate. Application to the synthesis of 6-methyl cyclopenta[*b*]pyridinone. *Tetrahedron* **2005**, *61* (19), 4569−4576.
(42) Lindstrom, M. J.; Bates, D. M. Nonlinear mixed effects models for repeated measures data. *Biometrics* **1990**, *46* (3), 673−687.
(43) Pinheiro J., B. D., DebRoy S., Sarkar D.; R Development Core Team. nlme: Linear and Nonlinear Mixed Effects Models. *R Package*, version 3; 2009, pp 1−96.
(44) Kaluz, S.; Kaluzova, M.; Stanbridge, E. J. Proteasomal inhibition attenuates transcriptional activity of hypoxia-inducible factor 1 (HIF-1) via specific effect on the HIF-1alpha C-terminal activation domain. *Mol. Cell. Biol.* **2006**, *26* (15), 5895−5907.