Novel ATP-Competitive Kinesin Spindle Protein Inhibitors

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Received April 12, 2007

Kinesin spindle protein (KSP), an ATPase responsible for spindle pole separation during mitosis that is present only in proliferating cells, has become a novel and attractive anticancer target with potential for reduced side effects compared to currently available therapies. We report herein the discovery of the first known ATP-competitive inhibitors of KSP, which display a unique activity profile as compared to the known loop 5 (L5) allosteric KSP inhibitors that are currently under clinical evaluation. Optimization of this series led to the identification of biphenyl sulfamide **20**, a potent KSP inhibitor with in vitro antiproliferative activity against human cells with either wild-type KSP (HCT116) or mutant KSP (HCT116 D130V). In a murine xenograft model with HCT116 D130V tumors, **20** showed significant antitumor activity following intraperitoneal dosing, providing in vivo proof-of-principle of the efficacy of an ATP-competitive KSP inhibitor versus tumors that are resistant to the other known KSP inhibitors.

Introduction

The kinesins are a family of motor proteins involved in a number of key cellular functions including subcellular organelle positioning, vesicular trafficking, and mitotic spindle assembly and function.[1−4] In general, these proteins couple the energy of ATP hydrolysis to a directed mechanical force along microtubules (MTs[a]). A sub-group of kinesins, the mitotic kinesins, are unique in that their expression is limited to the mitotic phase of cell division during which they act, often in complex with other proteins, to help establish the mitotic spindle, segregate and align chromosomes, and aid in cytokinesis.[5]

The mitotic spindle is a well-known and pharmaceutically validated target, and agents such as the taxanes (paclitaxel, docetaxel) and vinca alkaloids (vincristine, vinblastine, vinorelbine), which interfere with MT dynamics, are important chemotherapeutics currently in clinical use as anticancer agents (Chart 1).[6,7] However, because MTs are also present in other nondividing cells, including post-mitotic neurons, undesirable side effects, such as peripheral neuropathy, are often observed in patients undergoing treatment with these drugs.[8] In contrast, mitotic kinesins are absent from post-mitotic neurons, and small molecule inhibitors of these enzymes may lead to novel anticancer agents lacking the neuropathic side effects of the antitubulins.[9]

The mitotic kinesin family is comprised of at least 11 members with either overlapping or unique roles in mitosis.[10] Kinesin spindle protein (KSP or HsEg5) has recently been the focus of intense interest as a novel biological target for anticancer therapy. The role of this kinesin in mitosis has been elucidated using both biological (antibody microinjection, siRNA)[11,12] and small molecule inhibitor approaches.[13] KSP activity has been shown to be required early in mitosis for the establishment of a functional, bipolar mitotic spindle. In a number of human cancer cell lines, failure of spindle assembly has been associated with mitotic arrest, failure of the mitotic checkpoint, and subsequent apoptosis.

Following the discovery of monastrol (**1**, Figure 1),[13] several other small molecule KSP inhibitors have been reported from a variety of structural classes.[9,14−24] Of these, ispinesib (**2**) was the first compound to enter human clinical trials and is currently

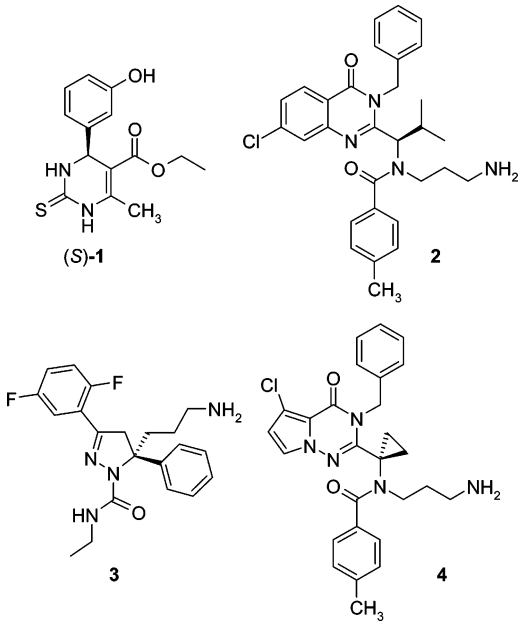

Figure 1. Some reported KSP inhibitors.

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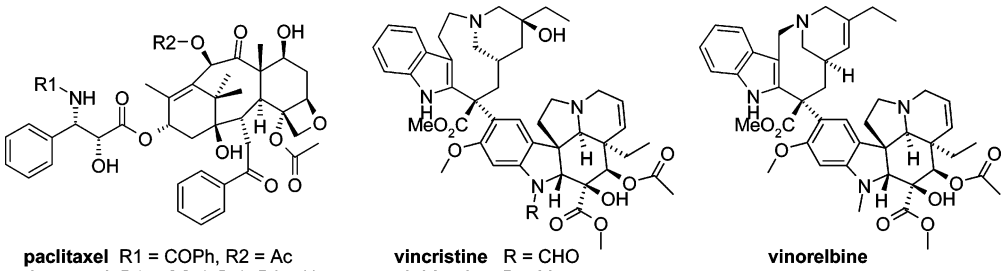
[a] Abbreviations: KSP, kinesin spindle protein; L5, loop 5; siRNA, small interfering RNA; MT, microtubule; SAR, structure−activity relationship; CTG, CellTiter-Glo; LHS, left-hand side; RHS, right-hand side; MTD, maximum tolerated dose; CR, complete regression; PR, partial regression.

10.1021/jm070435y CCC: $37.00
Published on Web 08/29/2007

Chart 1

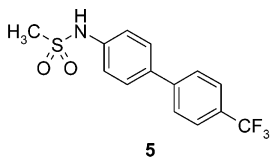


undergoing Phase II evaluation in various solid tumor types.[7,25] Ispinesib is a potent (subnanomolar K_i) KSP inhibitor with impressive in vivo efficacy in a variety of preclinical animal tumor xenograft models.[26,27] Biochemical and crystallographic characterization of **2** has established that the mechanism of action of this class of inhibitor is ATP-uncompetitive and MT-noncompetitive.[28] The compound binds to KSP at an induced fit binding site similar to that reported for **1**[29] and other inhibitors (**3**, **4**).[16,17,19,20]

Acquired resistance to chemotherapeutic agents as a result of either amino acid mutations of the target or at a cellular level by, for example, up-regulation of efflux mechanisms is a common occurrence. Indeed, the generation of a colorectal tumor cell line resistant to quinazolinone KSP inhibitors such as **2** has been reported (designated HCT116 D130V).[28] In these cells, DNA sequencing of KSP revealed a D130V mutation in the loop 5 (L5) region of the motor domain.[30,31] This region forms one side of a binding pocket shared by all of the currently reported KSP inhibitors (e.g., Figure 1). We reasoned that identification of KSP inhibitors that rely on accessing a distinct binding site and engaging different amino acids could represent a unique and complementary approach to the L5-targeted inhibitors. In addition, if this binding mode also resulted in a different mode of enzyme inhibition (e.g., ATP-competitive), it could allow for novel pharmacology because the association of KSP to MTs is strongly influenced by the occupancy of the ATP-binding pocket.[32]

To discover inhibitors with these properties, cell-active KSP inhibitors identified from a large library of compounds were screened versus the mutant (D130V) KSP. Chemically attractive hits were subsequently triaged using both the biochemical mode of inhibition studies as well as the cellular assays to rule out compounds with nonspecific or off-target effects.

The biphenyl methylsulfonamide **5** satisfied many of our criteria and was selected for further investigation (Figure 2). The biochemical potency of **5** (KSP K_i = 120 nM) was remarkable in view of its relatively simple structure and, under steady-state conditions, the compound was determined to be an ATP-competitive, MT-uncompetitive inhibitor (Figure 3). Although biphenyl **5** showed only modest activity in inhibiting the proliferation of human ovarian cancer cells (SKOV3 IC_{50} = 6 μM), analysis of inhibitor-treated SKOV3 cells by immunofluorescence confirmed a monopolar spindle phenotype, consistent with intracellular KSP inhibition. Importantly, the compound displayed similar potency against both wild-type KSP and mutant (D130V) KSP, suggesting that biphenyl **5** was interacting with KSP at a site distinct from the L5 inhibitor binding site. This article describes the synthesis and in vitro and in vivo characterization of a series of analogs of **5** displaying a unique mode of inhibition against KSP.



5

KSP	$K_i = 120 \pm 20$ nM
KSP D130V	$K_i = 110 \pm 10$ nM
SKOV3	$IC_{50} = 6128 \pm 1294$ nM

Figure 2. Novel biphenyl KSP inhibitor.

Results and Discussion

Chemistry. The relative simplicity of **5** as a lead was attractive because it offered an opportunity to modulate simultaneously both potency and developability properties. In addition, the ease of synthesis allowed for the rapid preparation of analogs to survey structure−activity relationship (SAR) trends. A general synthetic procedure for the preparation of various biphenyl analogs reported in this paper is outlined in Scheme 1. Functionalized biphenylamines were prepared via a Suzuki cross-coupling reaction between a bromoaniline and a phenyl boronic acid. Acylation of the aniline product with a sulfonyl chloride, isocyanate, sulfamoyl chloride, or carbamyl chloride provided several of the analogs described. Alternatively, acylation of a bromoaniline under similar conditions was carried out prior to Suzuki cross-coupling to yield the target biphenyl analogs directly. When performed in a parallel synthesis manner, these two complementary routes were sufficient to quickly access a large number of biphenyl analogs with varied substitution around the two halves (Tables 1 and 2).[33−35]

Biological Evaluation of Biphenyl Inhibitors. The ability of the biphenyl derivatives to inhibit KSP enzymatic activity was determined using the previously reported ATPase assay.[33,36] Biochemical mechanism of action studies, shown for **5** (Figure 3), were also performed on compounds **16**, **20**, **29**, and **30** and confirmed ATP-competitive inhibition (data not shown). Compounds were assayed for their antiproliferative activity against the human ovarian cancer cell line, SKOV3, using a luminescence-based readout (CTG). Finally, to assess the cellular phenotype resulting from inhibitor treatment, an automated cell imaging protocol was employed.

Initial studies established that major modifications to the biphenyl core, such as the introduction of one to three spacer atoms (e.g., O, S, C, N) between the two phenyl rings, were not tolerated (data not shown). This suggested that when bound to KSP, the molecule preferred to maintain a relatively linear conformation. We hypothesized that the methylsulfonamide moiety was important in making key hydrogen bond interactions, and this idea was supported by the large decrease in inhibitory activity observed when this group was moved to the *meta*-position (**6**, Table 1) or removed altogether (**7**). Similarly, methylation of the sulfonamide (**8**) or removal of the sulfonyl group to give methylamine **9** afforded analogs with decreased

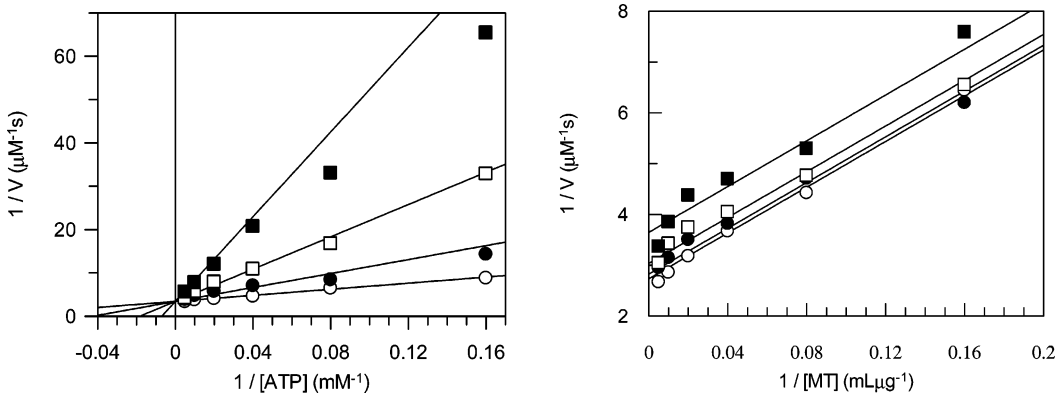
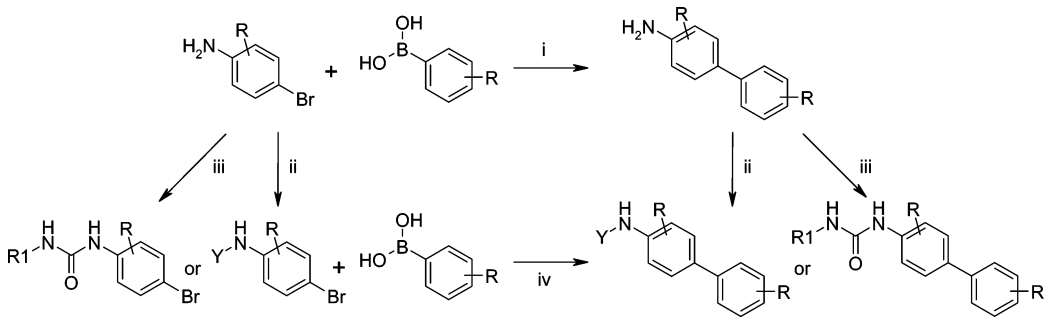


Figure 3. Mode of inhibition studies of biphenyl **5** to KSP by varying the ATP concentration in the presence of saturating MT (left) and by varying the MT concentration in the presence of saturating ATP (right). The Lineweaver−Burk plots indicate **5** is competitive with respect to ATP but uncompetitive with respect to MT.

Scheme 1[a]



[a] Reagents and conditions: (i) $Pd(PPh_3)_4$ (cat.), aq K_2CO_3 soln, DMF, 100 °C; (ii) Y−Cl (Y = SO_2R^1, $SO_2NR^1R^2$, or $CONR^1R^2$); (iii) R^1—N═C═O, CH_2Cl_2; (iv) $PdCl_2$(dppf) (cat.), aq Na_2CO_3 soln, CH_3CN, μwave, 100 °C.

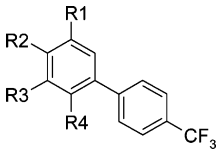
biochemical potency, highlighting a need for a hydrogen bond donor and potentially a hydrogen bond acceptor. Extending the methyl group of the sulfonamide to an ethyl (**10**) or elaborating it further (data not shown) also led to large decreases in KSP inhibitory activity, consistent with a high degree of steric constraint in this region.

Replacement of the methylsulfonamide moiety led to biphenyl analogs encompassing a wider range of inhibitory activity. While some polar groups with hydrogen bond donating capabilities (**11**, **12**) had modest KSP inhibitory activity, others were at best equipotent to **5** (e.g., **13**). Better results were obtained when the methylsulfonamide in **5** was replaced with a urea moiety (**16**), giving a compound that had approximately 4-fold higher potency in inhibiting KSP. This increased biochemical potency also translated to improved cellular activity (Table 2). To probe further the SAR of the urea group, the isosteric thiourea (**17**) and guanidine (**18**) analogs were prepared. Although the basic guanidine **18** lacked inhibitory activity, the thiourea **17** led to another 4-fold increase in biochemical potency. We therefore prepared the corresponding thiourea bioisostere, cyanoguanidine **19**.[37] This analog had measurable KSP inhibitory activity but was approximately 20-fold less potent than the urea (**16**) and 80-fold less potent than **17**. Substitution of the urea thiocarbonyl moiety with a sulfonyl group proved to be more feasible and provided the sulfamide **20**, which was approximately equipotent to **17**.

Methylation or substitution of the various hydrogen bond donors in the urea and sulfamide analogs was subsequently investigated (**21**−**28**). Mono- or bismethylation on the terminal nitrogen led to analogs with significantly decreased KSP inhibitory activity, potentially the result of substantial steric constraint, as observed in earlier attempts to extend the methylsulfonamide (e.g., **5** to **10**). However, analogs predicted to have a similar pK_a for the anilino nitrogen (e.g., **20** and **23**)[38] also showed a 100-fold difference in KSP inhibition, perhaps reflecting the greater importance of the terminal amino group as a weakly acidic hydrogen bond donor. Consistent with this hypothesis, methylation of the anilino nitrogen (**24**) or replacement with a methylene (**25**) or oxygen (**27**) led to analogs with reasonable to very good KSP inhibitory activity. Interestingly, perhaps indicative of subtle differences between the urea and the sulfamide subseries, sulfamate **27** showed only a 3-fold decrease in potency as compared to the parent sulfamide (**20**), in contrast to the 18-fold decrease observed when introducing the same substitution in the ureas (i.e., carbamate **28** compared to the parent urea **16**).

With the identification of the sulfamide as an effective methylsulfonamide replacement, we proceeded to evaluate the effects of additional substituents on the left phenyl ring (LHS), with an aim to increase potency further. Sterically undemanding substituents *ortho* to the urea or sulfamide were tolerated, with the presence of a fluorine atom providing a more potent KSP inhibitor (**29**, **30**). As noted above, this effect appeared to be unrelated to the enhanced acidity of the sulfamide group, as indicated by the decreased potency of analogs with more electron-withdrawing groups (**31**, **37**, **38**, **39**).[38] Replacement of the fluorine atom (e.g., **32**, **34**, **35**, **37**) provided biphenyl analogs with good to excellent KSP inhibitory activity, with the primary amino group (**35**) providing a compound with the best activity of the fluorine replacements. Based on a 60-fold reduction in potency of the methylamine **36** compared to the corresponding primary amine **35**, it seemed likely that either both anilino hydrogens were involved in making critical interactions with KSP or the binding pocket could not favorably accommodate the additional methyl group. However, the unexpectedly good activity of methyl ether **33**, an isosteric

Table 1. KSP Inhibitory Activity of LHS Analogs

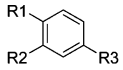


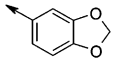
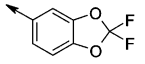
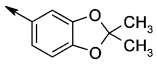
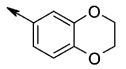
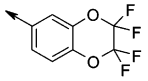
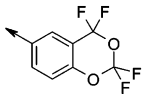
cmpd	R1	R2	R3	R4	KSP IC_{50} (nM)
5	H	$NHSO_2CH_3$	H	H	161
6	$NHSO_2CH_3$	H	H	H	6000
7	H	H	H	H	5320
8	H	$N(CH_3)SO_2CH_3$	H	H	>20 000
9	H	$NHCH_3$	H	H	3726
10	H	$NHSO_2CH_2CH_3$	H	H	2518
11	H	NH_2	H	H	1649
12	H	OH	H	H	700
13	H	SO_2NH_2	H	H	345
14	H	$C(O)NH_2$	H	H	>20 000
15	H	CO_2H	H	H	>20 000
16	H	$NHC(O)NH_2$	H	H	39
17	H	$NHC(S)NH_2$	H	H	11
18	H	$NHC(NH)NH_2$	H	H	>20 000
19	H	$NHC(NCN)NH_2$	H	H	840
20	H	$NHSO_2NH_2$	H	H	18
21	H	$NHC(O)NHCH_3$	H	H	>20 000
22	H	$NHC(O)N(CH_3)_2$	H	H	>20 000
23	H	$NHSO_2N(CH_3)_2$	H	H	1795
24	H	$N(CH_3)SO_2NH_2$	H	H	1422
25	H	$CH_2SO_2NH_2$	H	H	118
26	H	$CH_2SO_2NHCH_3$	H	H	>20 000
27	H	OSO_2NH_2	H	H	64
28	H	$OC(O)NH_2$	H	H	716
29	H	$NHC(O)NH_2$	F	H	15
30	H	$NHSO_2NH_2$	F	H	5
31	H	$NHSO_2NH_2$	CN	H	196
32	H	$NHSO_2NH_2$	CH_3	H	141
33	H	$NHSO_2NH_2$	OCH_3	H	66
34	H	$NHSO_2NH_2$	OH	H	349
35	H	$NHSO_2NH_2$	NH_2	H	24
36	H	$NHSO_2NH_2$	$NHCH_3$	H	1448
37	H	$NHSO_2NH_2$	Cl	H	67
38	H	$NHSO_2NH_2$	H	F	685
39	F	$NHSO_2NH_2$	F	H	502

analog of **36** that lacks hydrogen bond donating capabilities, suggested that the binding site may be flexible enough to compensate for alternative key interactions. Substitution close to the biphenyl juncture at R4 with even a fluorine atom (**38**) led to a significant decrease in KSP inhibitory potency, indicating the binding site may be sensitive to the planarity of the molecule.

Having established that urea or sulfamide were close to being optimal groups on the left phenyl for KSP inhibition, we next investigated expanding the SAR around the right phenyl ring (RHS) of the biphenyl core. It quickly became apparent that there was a very high preference for a trifluoromethyl group in the *para*-position (Table 2). Removal (**44**), transposition to the *meta*-position (**45**), or replacement of the trifluoromethyl group (**16**) with the smaller methyl group (**46**) afforded analogs either devoid of or with significantly less KSP affinity. Nonetheless, replacements such as an isopropyl (**47**) or a *t*-butyl (**48**) were better tolerated, particularly in the biochemical KSP inhibition assay. This suggested that the molecular volume associated with the trifluoromethyl group rather than the electronic nature of the group was the more critical element in imparting activity. Reasons for the approximately 7-fold reduction in cellular activity are unclear, as the analogs, especially **47**, which displays a similar clogP,[39] encompass similar permeability and solubility properties as the parent (**16**). The use of a halogen (**40**, **49**) in

Table 2. Biochemical and Cellular Data for RHS Analogs



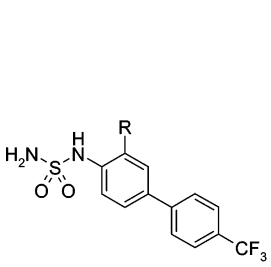
cmpd	R1	R2	R3	KSP IC_{50} (nM)	SKOV3 IC_{50} (nM)[a]
5	$NHSO_2CH_3$	H	4-CF_3-Ph	161	6128
40	$NHSO_2CH_3$	H	4-Cl-Ph	2002	>20,000
41	$NHSO_2CH_3$	H	4-CH_2CF_3-Ph	>20,000	NT
42	$NHSO_2CH_3$	H	4-OCF_3-Ph	792	>5,000
43	$NHSO_2CH_3$	H	4-SO_2CF_3-Ph	176	11,234
16	$NHC(O)NH_2$	H	4-CF_3-Ph	39	759
44	$NHC(O)NH_2$	H	Ph	>20,000	NT
45	$NHC(O)NH_2$	H	3-CF_3-Ph	7200	>20,000
46	$NHC(O)NH_2$	H	4-CH_3-Ph	1382	>20,000
47	$NHC(O)NH_2$	H	4-$CH(CH_3)_2$-Ph	57	5259
48	$NHC(O)NH_2$	H	4-$C(CH_3)_3$-Ph	54	5354
49	$NHC(O)NH_2$	F	3-F-4-Br-Ph	262	9954
50	$NHC(O)NH_2$	H	4-SCF_3-Ph	240	11,056
51	$NHC(O)NH_2$	H	4-SO_2CF_3-Ph	58	1978
52	$NHC(O)NH_2$	F	4-$SO_2CH_2CH_3$-Ph	10,904	>20,000
53	$NHC(O)NH_2$	H	4-$C(O)CF_3$-Ph	>20,000	NT
20	$NHSO_2NH_2$	H	4-CF_3-Ph	18	162
54	$NHSO_2NH_2$	H	3-F-4-CF_3-Ph	15	136
55	$NHSO_2NH_2$	H	3-NO_2-4-CF_3-Ph	28	1176
56	$NHSO_2NH_2$	H	3-NH_2-4-CF_3-Ph	167	3821
57	$NHSO_2NH_2$	H		7682	>20,000
58	$NHSO_2NH_2$	H		31	867
59	$NHSO_2NH_2$	H		482	>14,600
60	$NHSO_2NH_2$	H		>20,000	NT
61	$NHSO_2NH_2$	H		67	6748
62	$NHSO_2NH_2$	H		319	12,992

[a] NT = not tested.

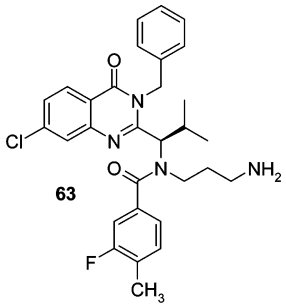
lieu of the trifluoromethyl group led to more modest inhibitors in the biochemical KSP assay.

Since electronegative fluorine atoms have been reported as weak hydrogen bond acceptors,[40] we investigated this region for such effects. Homologation of the trifluoromethyl group by a methylene (**41**) provided an analog with negligible KSP inhibitory activity. However, homologation by either an oxygen (**42**) or sulfur (**50**) atom was tolerated, although each led to some decrease in biochemical activity as compared to **5**. Furthermore, conversion of **50** to the corresponding sulfone (**51**) led to a more potent inhibitor, exhibiting similar biochemical

Table 3. Biological Evaluation of Optimized Biphenyls vs Wild-Type and Mutant KSP



20 R = H
30 R = F



63

cmpd	WT KSP K_i (nM)	D130V KSP K_i (nM)	HCT116 IC_{50} (nM)	HCT116 D130V IC_{50} (nM)
20	6.2 ± 0.4	7 ± 1	403 ± 225	5.4 ± 1.5[a]
30	3.5 ± 0.2	6 ± 1	294 ± 151	3.1 ± 0.8[a]
63	<1	350 ± 10	2.0 ± 1.0	1372 ± 631

[a] Data from one experiment (run in duplicate).

and cellular potency to the parent trifluoromethyl analog (**16**). The increased potency of sulfone **51** over thioether **50** was not merely due to the presence of the oxygen atoms, as evidenced by the lack of activity of ethylsulfone **52**, an analog that would have been expected to show moderate activity based on the ability to replace the trifluoromethyl group with alkyl groups. To mimic the electron-withdrawing effect of the sulfone, the corresponding trifluoromethylketone **53** was prepared but, surprisingly, lacked KSP inhibitory activity. Such electron-deficient ketones are known to readily form hydrates in aqueous media,[41,42] and the resulting ketal is presumably not compatible with KSP inhibition. The incorporation of additional groups capable of participating in hydrogen bonds, such as a carboxylic acid, carboxamide, methyl ether, methyl ketone, or phosphonate, afforded compounds without significant KSP activity (data not shown).

As with the left-hand side, additional substitution on the right-hand side ring was interrogated, with substitution near the biphenyl junction again being detrimental to KSP inhibitory activity. However, substitution *ortho* to the trifluoromethyl group was tolerated, although the substituent range investigated was small. With a fluorine atom (**54**), both biochemical and cellular potencies were similar to the parent (**20**), whereas the nitro analog (**55**) showed similar biochemical but decreased cellular activity. Reduction of the nitro group to an amine (**56**) led to a reduction in both biochemical and cellular potency. Interestingly, cyclization of the 3,4-position to afford benzodioxoles or benzodioxins led to analogs showing good KSP inhibitory activity only when fluorinated (**58**, **61**, **62**), although the cellular activity of these analogs was diminished as compared to the monocyclic parent (**20**). Generally, the presence of fluorine atoms in this region potentiated biochemical activity, indicating that an electronic contribution from the alkylfluorides could not be excluded (e.g., **57** vs **58**, **60** vs **61**). However, despite our efforts at modification, the balance of electronic and steric properties of the trifluoromethyl group uniquely seemed to provide analogs with the best biochemical and cellular activity.

Although in general the lead inhibitor **5** was resistant to a variety of structural changes, we were able to increase significantly the KSP inhibitory activity as well as introduce cellular activity to the compound by making smaller, targeted modifications. Two of the most potent compounds identified from this investigation (**20**, **30**) were evaluated for their activity versus the mutant form of KSP (KSP D130V). As shown in Table 3, both compounds were highly potent enzyme inhibitors and did not discriminate between wild-type and mutant KSP. In contrast, quinazolinone **63**,[14] an analog of **2**, was more than 350-fold more potent versus wild-type compared to the mutant enzyme. As expected, **63** was far less effective in inhibiting KSP containing the D130V mutation in the L5 region where this compound binds. This profile of activity was also reproduced in cell culture where sulfamides **20** and **30** both showed antiproliferative activity versus wild-type (HCT116) and mutant (HCT116 D130V) KSP cell lines, whereas **63** was poorly active in cells containing the L5-mutated KSP. Surprisingly, sulfamides **20** and **30** were of much higher potency in inhibiting the proliferation of the mutant cell line relative to the wild-type cell line. We rationalized that this could be due to differences in the catalytic properties between the mutant and the wild-type KSP enzymes. Indeed, biochemical characterization of the KSP D130V enzyme showed that the K_m for ATP was approximately 20-fold higher than that for wild-type KSP.[43] Because both **20** and **30**, like **5**, are ATP-competitive inhibitors, the reduced affinity of the mutant enzyme toward ATP would have the effect of increasing its sensitivity toward the biphenyl inhibitors.[44,45]

Table 4. Pharmacokinetic Profile of **20** in the Conscious Nude Mouse

route	C_{max} (μg/mL)	C_{last}[a] (μg/mL)	$DNAUC_{(0-t)}$ (μg h/mL/mg/kg)
ip	10.49	8.73	2.35

[a] Taken at 24 h.

In Vivo Evaluation of Biphenyl Inhibitors. In view of its exquisite cellular potency against the HCT116 D130V colorectal tumor line, sulfamide **20** made an excellent candidate for in vivo evaluation using this cell line in a xenograft model. Prior to in vivo experiments, the pharmacokinetic profile of **20** was determined (Table 4). Following ip administration at a nontoxic dose (82 mg/kg), sulfamide **20** led to a maximum concentration in plasma of 10.49 μg/mL that was sustained for 24 h (C_{last} = 8.73 μg/mL). Although the elimination half-life could not be estimated, the data indicate sulfamide **20** exhibits a long half-life and good exposure at biologically active doses in the mouse (vide infra).

Sulfamide **20** was evaluated for antitumor activity against advanced HCT116 D130V solid tumors implanted in nude mice in doses ranging from 16 mg/kg up to 125 mg/kg delivered ip every 4 days for a total of three doses (Figure 4). Relative to placebo-treated animals, tumors in mice treated with **20** showed either regressions or significant tumor growth delay. Indeed, at the highest dose tested (MTD > 125 mg/kg), complete tumor regressions (CR) were observed in three of five animals dosed, whereas at lower doses, tumor growth delays of at least 19 days (16 mg/kg dose) were observed. In contrast and as expected due to its decreased activity in cells carrying the mutant KSP, the quinazolinone **63** showed only a modest delay in tumor growth (10 days) under similar conditions at its MTD (10 mg/kg). These results demonstrate for the first time that an ATP-competitive KSP inhibitor can induce significant antitumor effects and can overcome resistance due to binding site mutations generated in vitro by allosteric quinazolinone KSP inhibitors.

The in vivo efficacy of **20** versus tumor cell lines carrying wild-type KSP was also investigated using the Colo205 colorectal tumor cell line, historically one of the more sensitive cell lines to KSP inhibitors. In marked contrast to its activity versus tumor lines bearing a mutant KSP, sulfamide **20** did not show significant antitumor activity at doses up to its MTD (20 mg/kg) versus Colo205 tumors that contain wild-type KSP

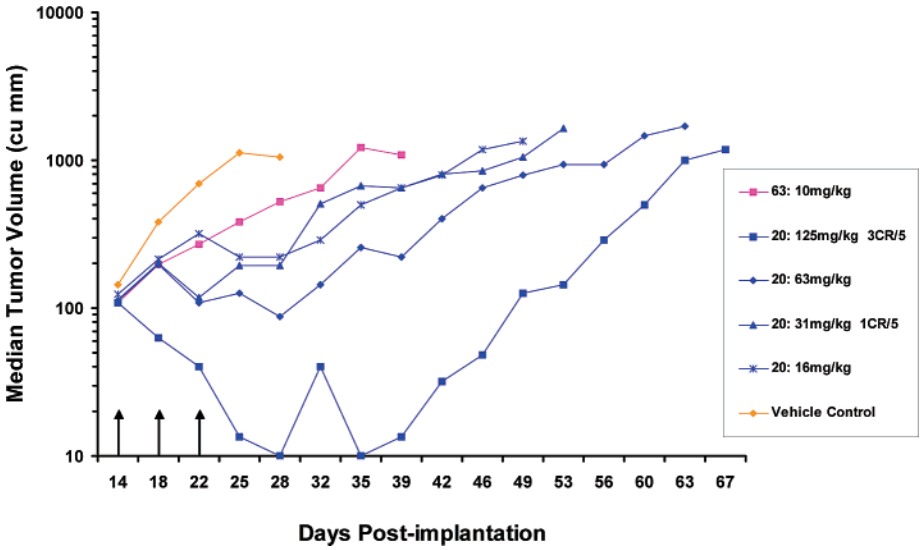


Figure 4. In vivo efficacy of **20** against advanced HCT116 D130V xenografts in nude mice (dosed ip q4dx3, indicated by arrows).

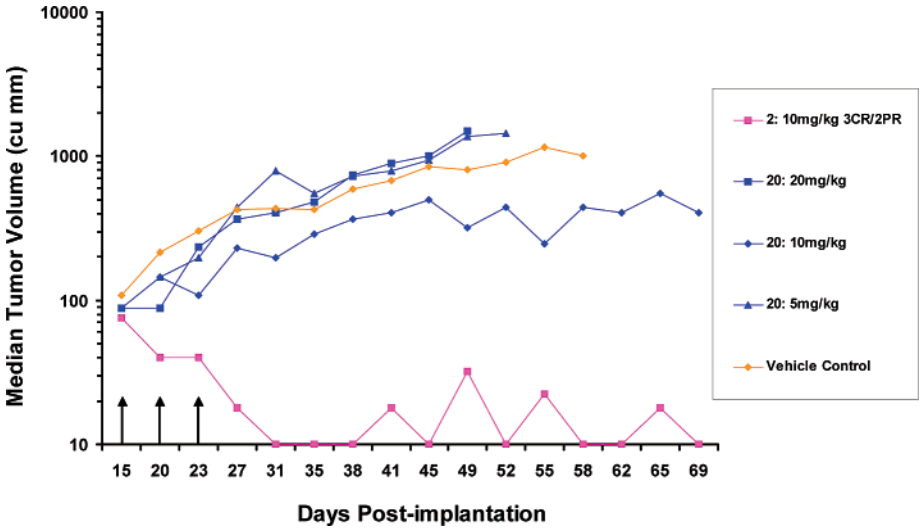


Figure 5. In vivo efficacy of **20** against advanced Colo205 xenografts in nude mice (dosed ip q4dx3, indicated by arrows). Dosage of **20** at 40 mg/kg was toxic.

(Figure 5). In line with its superior activity relative to **20** in wild-type cell culture, the quinazolinone **2** was very effective, leading to three complete and two partial (PR) tumor regressions of five animals dosed. The lack of efficacy for **20** in a wild-type model versus a mutant-KSP model may be rationalized by its more modest potency in Colo205 cells (IC_{50} = 181 nM) as compared to HCT116 D130V cells (IC_{50} = 5.4 nM). These results suggest that in vivo efficacy with ATP-competitive inhibitors versus a tumor cell line carrying wild-type KSP may require substantially more potent inhibitors to overcome the high cellular ATP concentration, and further studies directed toward this will be reported in due course.

Conclusion

We have identified a novel biphenyl series of KSP inhibitors that display good biochemical and cellular activity and exhibit a unique mode of action. These ATP-competitive inhibitors contrast with the previously reported ATP-uncompetitive class of KSP inhibitors both in mechanism and in activity versus KSP D130V, the L5 mutant KSP generated in cell culture with resistance to a quinazolinone inhibitor. All data are consistent with a binding site distinct from the previously reported L5 allosteric KSP inhibitors. Whereas previous inhibitors, exemplified here by the quinazolinones, are significantly less effective at inhibiting KSP containing select point mutations in the L5 region, the ATP-competitive biphenyls are potent inhibitors of KSP D130V and represent a way to overcome resistance generated by the L5 binders. Indeed, the biphenyl analogs have demonstrated antitumor activity in an HCT116 D130V mouse xenograft model. Although the reported inhibitors could be useful to treat emergent L5-mutated tumors, it would be more desirable to develop ATP-competitive KSP inhibitors with efficacy versus both wild-type and mutant KSP tumors. The development and characterization of more potent ATP-competitive KSP inhibitors will be the subject of future publications.

Experimental Section

Chemistry. General Methods. Unless otherwise noted, starting materials and reagents were purchased from commercial sources and used without further purification. Air- or moisture-sensitive reactions were carried out under a nitrogen atmosphere. Anhydrous solvents were obtained from Sigma-Aldrich. Microwave irradiation was carried out in a Personal Chemistry Emrys Optimizer microwave. Flash chromatography was performed using silica gel (EM Science, 230−400 mesh) under standard techniques or using silica gel cartridges (RediSep normal phase disposable flash columns) on an Isco CombiFlash. Reverse phase HPLC purification was

conducted on a Gilson HPLC (monitoring at a wavelength of 214 or 254 nm) with a YMC ODS-A C18 column (5 μm, 75 × 30 mm), eluting with 5−90% CH_3CN in H_2O with 0.1% TFA. ^1H NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer. Chemical shifts (δ) are reported in ppm relative to an internal solvent reference. Apparent peak multiplicities are described as s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), or m (multiplet). Coupling constants (J) are reported in hertz (Hz) after the integration. Mass spectra were recorded on an Applied Biosystems MSD Sciex API 150EX single quadrupole mass spectrometer with an electrospray ionization (ESI) source. Analytical HPLC was conducted on an Agilent 1100 Series HPLC with a Zorbax Eclipse XDB-C18 column (5 μm, 4.6 × 150 mm) using a 10 min gradient (10−90% CH_3CN/H_2O with 0.1% TFA). The retention time (t_R) is expressed in min at a UV detection of 254 nm. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ.

General Method for Suzuki Cross-Coupling. Method A: 4′-(Trifluoromethyl)-4-biphenylamine (11). A solution of 4-bromoaniline (29 mmol), 4-trifluoromethylphenyl boronic acid (35 mmol), and tetrakis(triphenylphosphine)palladium(0) (1.4 mmol) in 2 M aq potassium carbonate (50 mL) and DMF (50 mL) was heated at 100 °C for 17 h. The reaction mixture was cooled, poured into half-saturated aq sodium bicarbonate (400 mL), and extracted with (3 × 400 mL) diethyl ether. The combined organic layers were dried over sodium sulfate and concentrated in vacuo. Purification of the residue by silica gel chromatography (10−30% ethyl acetate/hexanes) provided the title product as a white powder (70%). ^1H NMR (DMSO-d_6): δ 7.76 (d, 2H, J = 8.1 Hz), 7.69 (d, 2H, J = 8.3 Hz), 7.45 (d, 2H, J = 8.6 Hz), 6.67 (d, 2H, J = 8.6 Hz), 5.41 (s, 2H). MS m/z 238.2 $[M + H]^+$. Analytical HPLC 98.5% purity, t_R = 5.39.

General Method for Sulfonylation. Method B: *N*-[4′-(Trifluoromethyl)-4-biphenylyl]methanesulfonamide (5). To a solution of 4′-(trifluoromethyl)-4-biphenylamine (0.42 mmol) in dichloromethane (2.0 mL) was sequentially added pyridine (0.84 mmol) and methanesulfonylchloride (0.63 mmol). The reaction mixture was stirred at room temperature for 18 h and then concentrated in vacuo. Purification by reverse phase HPLC afforded the title product as a white solid (47%). ^1H NMR (DMSO-d_6): δ 9.99 (s, 1H), 7.88 (d, 2H, J = 8.2 Hz), 7.81 (d, 2H, J = 8.4 Hz), 7.75 (d, 2H, J = 8.6 Hz), 7.34 (d, 2H, J = 8.6 Hz), 3.06 (s, 3H). MS m/z 316.2 $[M + H]^+$. Anal. ($C_{14}H_{12}F_3NO_2S$) C, H, N.

***N*-[4′-(Trifluoromethyl)-3-biphenylyl]methanesulfonamide (6).** The intermediate 4′-(trifluoromethyl)-3-biphenylamine was prepared following Method A using 3-bromoaniline (83%). ^1H NMR ($CDCl_3$): δ 7.59 (s, 3H), 7.19 (m, 2H), 6.91 (dd, 1H, J = 7.7, 1.6 Hz), 6.83 (t, 1H, J = 1.9 Hz), 6.66 (dd, 1H, J = 7.9, 2.3 Hz), 3.72 (s, 2H). MS m/z 238.2 $[M + H]^+$. The title compound was prepared from this aniline following Method B (45%). ^1H NMR (DMSO-d_6): δ 9.92 (s, 1H), 7.85 (s, 4H), 7.52-7.47 (m, 3H), 7.29 (d, 1H, J = 6.8 Hz), 3.06 (s, 3H). MS m/z 316.2 $[M + H]^+$. Anal. ($C_{14}H_{12}F_3NO_2S$) C, H, N.

4-(Trifluoromethyl)biphenyl (7). The title compound was prepared as a white solid following Method A using 4-bromobenzene (55%). ^1H NMR (DMSO-d_6): δ 7.91 (d, 2H, J = 8.2 Hz), 7.83 (d, 2H, J = 8.3 Hz), 7.75 (d, 2H, J = 7.1 Hz), 7.53 (m, 2H), 7.45 (t, 1H, J = 7.3 Hz). MS m/z 223.2 $[M + H]^+$. Analytical HPLC 97.3% purity, t_R = 9.78.

***N*-Methyl-*N*-[4′-(trifluoromethyl)-4-biphenylyl]methanesulfonamide (8).** Sodium hydride (0.15 mmol of a 60% dispersion in mineral oil) was carefully added to a solution of *N*-[4′-(trifluoromethyl)-4-biphenylyl]methanesulfonamide (0.13 mmol) in DMF (2 mL). After 20 min, methyl iodide (0.19 mmol) was added, and the reaction mixture was stirred at room temperature for 16 h. The reaction mixture was poured into water (75 mL) and extracted with (3 × 70 mL) ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated in vacuo. Purification of the residue by silica gel chromatography (10−35% ethyl acetate/hexanes) provided the title product as a white solid (quantitative). ^1H NMR (DMSO-d_6): δ 7.92 (d, 2H, J = 8.1 Hz), 7.83 (d, 2H, J = 8.5 Hz), 7.79 (d, 2H, J = 8.6 Hz), 7.55 (d, 2H, J = 8.6 Hz), 3.29 (s, 3H), 3.00 (s, 3H). MS m/z 330.0 $[M + H]^+$. Anal. ($C_{15}H_{14}F_3NO_2S$) C, H, N.

Methyl[4′-(trifluoromethyl)-4-biphenylyl]amine (9). To a solution of 4′-(trifluoromethyl)-4-biphenylamine (4.21 mmol) and pyridine (6.31 mmol) in dichloromethane (30 mL) was added 4-nitrophenyl formate (4.21 mmol). The reaction mixture was stirred at room temperature for 18 h. The reaction mixture was dissolved in ethyl acetate (150 mL) and washed with water (3 × 100 mL) and brine (1 × 100 mL). The organic layer was dried over sodium sulfate and was concentrated in vacuo. Purification of the residue by reverse phase HPLC afforded the intermediate [4′-(trifluoromethyl)-4-biphenylyl]formamide as a white solid (85%). MS m/z 266.2 $[M + H]^+$. A solution of this formamide (3.75 mmol) in 1 M lithium aluminum hydride in tetrahydrofuran (10 mL) was stirred for 18 h at room temperature. The reaction was quenched with saturated sodium sulfate solution (10 mL), extracted into ethyl acetate (3 × 10 mL), and concentrated in vacuo. The residue was purified on reverse phase HPLC to afford the title product as a brown solid (15%). ^1H NMR (DMSO-d_6): δ 7.78 (d, 2H, J = 8.0 Hz), 7.70 (d, 2H, J = 8.0 Hz), 7.55 (d, 2H, J = 8.0 Hz), 6.70 (d, 2H, J = 8.0 Hz), 2.74 (s, 3H). MS m/z 252.2 $[M + H]^+$. Analytical HPLC 94.5% purity, t_R = 6.16.

***N*-[4′-(Trifluoromethyl)-4-biphenylyl]ethanesulfonamide (10).** The title compound was prepared following Method B using ethanesulfonylchoride (88%). ^1H NMR (DMSO-d_6): δ 10.02 (s, 1H), 7.86 (d, 2H, J = 8.3 Hz), 7.79 (d, 2H, J = 8.3 Hz), 7.72 (d, 2H, J = 8.6 Hz), 7.34 (d, 2H, J = 8.6 Hz), 3.15 (q, 2H, J = 7.3 Hz), 1.21 (t, 3H, J = 7.3 Hz). MS m/z 330.0 $[M + H]^+$. Anal. ($C_{15}H_{14}F_3NO_2S$) C, H, N.

4′-(Trifluoromethyl)-4-biphenylol (12). The title compound was prepared as a white solid following Method A using 4-bromophenol with purification by reverse phase HPLC (46%). ^1H NMR (DMSO-d_6): δ 9.75 (s, 1H), 7.81 (d, 2H, J = 8.4 Hz), 7.75 (d, 2H, J = 8.5 Hz), 7.58 (dd, 2H, J = 6.7, 1.9 Hz), 6.89 (dd, 2H, J = 6.7, 1.9 Hz). MS m/z 239.2 $[M + H]^+$. Analytical HPLC 99.6% purity, t_R = 7.77.

4′-(Trifluoromethyl)-4-biphenylsulfonamide (13). The title compound was prepared as a yellow solid following Method A using 4-bromobenzenesulfonamide with purification by reverse phase HPLC (50%). ^1H NMR (DMSO-d_6): δ 7.76 (d, 2H, J = 8.4 Hz), 7.64 (d, 2H, J = 8.6 Hz), 7.52 (d, 2H, J = 8.8 Hz), 7.48 (d, 2H, J = 8.6 Hz), 7.23 (s, 2H). MS m/z 302.2 $[M + H]^+$. Anal. ($C_{13}H_{10}F_3NO_2S$) C, H, N.

4′-(Trifluoromethyl)-4-biphenylcarboxamide (14). The title compound was prepared as a white solid following Method A using 4-bromobenzamide with purification by reverse phase HPLC (35%). ^1H NMR (DMSO-d_6): δ 8.08 (s, 1H), 8.01 (d, 2H, J = 8.6 Hz), 7.97 (d, 2H, J = 8.1 Hz), 7.86-7.83 (m, 4H), 7.46 (s, 1H). MS m/z 266.2 $[M + H]^+$. Anal. ($C_{14}H_{10}F_3NO$) C, H, N.

4′-(Trifluoromethyl)-4-biphenylcarboxylic Acid (15). The title compound was prepared following Method A using 4-bromobenzoic acid with purification by reverse phase HPLC (6%). ^1H NMR (DMSO-d_6): δ 12.10 (s, 1H), 8.07 (d, 2H, J = 8.4 Hz), 7.98 (d, 2H, J = 8.2 Hz), 7.88 (m, 4H). MS m/z 267.2 $[M + H]^+$. Anal. ($C_{14}H_9F_3O_2$) C, H.

General Method for Urea Formation. Method C: *N*-[4′-(Trifluoromethyl)-4-biphenylyl]urea (16). To a solution of 4′-(trifluoromethyl)-4-biphenylamine (0.84 mmol) in dichloromethane (10.0 mL) was added chlorosulfonylisocyanate (1.3 mmol). The reaction mixture was stirred at room temperature for 3 h, during which time a white precipitate formed. The reaction mixture was quenched with water (10.0 mL) and stirred 18 h. The mixture was filtered, washed with water, and the solid was dried in vacuo. Recrystallization of the solid from hot isopropanol afforded the title compound as a white powder (54%). Alternatively, the product could be purified by reverse phase HPLC or by flash chromatography (50−90% ethyl acetate/hexanes). ^1H NMR (DMSO-d_6): δ 8.73 (s, 1H), 7.85 (d, 2H, J = 8.3 Hz), 7.77 (d, 2H, J = 8.4 Hz),

7.64 (d, 2H, $J = 8.7$ Hz), 7.54 (d, 2H, $J = 8.7$ Hz), 5.94 (s, 2H). MS *m*/*z* 281.2 $[M + H]^+$. Analytical HPLC 97.7% purity, $t_R = 6.53$.

***N*-[4′-(Trifluoromethyl)-4-biphenylyl]thiourea (17).** A solution of 4′-(trifluoromethyl)-4-biphenylamine (0.84 mmol) in water (1 mL) and 1 M hydrochloric acid solution (0.84 mL) was treated with ammonium thiocyanate (0.84 mmol). The reaction mixture was heated at 110 °C for 2 h. The homogeneous reaction solution was cooled and poured onto ice (4 g). A white precipitate formed and was collected by filtration. Purification of the residue by reverse phase HPLC yielded the title product (21%). ^{1}H NMR (CD_3CN): δ 8.56 (s, 1H), 7.93 (d, 2H, $J = 8.6$ Hz), 7.87 (d, 2H, $J = 8.6$ Hz), 7.81 (d, 2H, $J = 8.6$ Hz), 7.60 (d, 2H, $J = 8.6$ Hz), 6.57 (br s, 2H). MS *m*/*z* 297.5 $[M + H]^+$. Analytical HPLC 87.8% purity, $t_R = 6.86$.

***N*-[4′-(Trifluoromethyl)-4-biphenylyl]guanidine (18).** A solution of 4′-(trifluoromethyl)-4-biphenylamine (0.30 mmol) in pyridine (2.0 mL) was treated with 1*H*-pyrazole-1-carboxamidine hydrochloride (0.32 mmol). The reaction mixture was stirred at room temperature for 19 h, heated at 50 °C for 22 h, and then heated at 100 °C for 7 h. The mixture was cooled, poured into water (50 mL) and brine (20 mL), and extracted with (3 × 60 mL) ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated in vacuo. Purification of the residue by reverse phase HPLC provided the trifluoroacetate salt of the title product as a white solid (26%). ^{1}H NMR (DMSO-d_6): δ 9.91 (s, 1H), 7.92 (d, 2H, $J = 8.2$ Hz), 7.84 (m, 4H), 7.58 (br s, 3H), 7.38 (d, 2H, $J = 8.6$ Hz). MS *m*/*z* 280.2 $[M + H]^+$. Anal. ($C_{14}H_{12}F_3N_3 \cdot C_2HF_3O_2$) C, H, N.

***N*-Cyano-*N*′-[4′-(trifluoromethyl)-4-biphenylyl]guanidine (19).** A solution of 4′-(trifluoromethyl)-4-biphenylamine (0.32 mmol) and sodium dicyanamide (0.35 mmol) in water (2.0 mL) with 1 N aq hydrochloric acid (0.35 mmol) was heated at 60 °C for 5.5 h. The reaction mixture was poured into water (55 mL) and brine (15 mL) and extracted with (3 × 50 mL) ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated in vacuo. Purification of the residue by silica gel chromatography (60−100% ethyl acetate/hexanes) afforded the title product as an ivory solid (26%). ^{1}H NMR (DMSO-d_6): δ 9.25 (s, 1H), 7.87 (d, 2H, $J = 8.1$ Hz), 7.80 (d, 2H, $J = 8.3$ Hz), 7.71 (d, 2H, $J = 8.9$ Hz), 7.51 (d, 2H, $J = 8.9$ Hz), 7.12 (s, 2H). MS *m*/*z* 305.2 $[M + H]^+$. Analytical HPLC 97.5% purity, $t_R = 6.76$.

General Method for Sulfamide Formation. Method D: *N*-[4′-(Trifluoromethyl)-4-biphenylyl]sulfamide (20). To an ice-cooled solution of chlorosulfonylisocyanate (0.47 mmol) in acetonitrile (1.0 mL) was added water (0.47 mmol). The reaction mixture was stirred at 0 °C for 1 min and then allowed to warm to room temperature slowly. After 3 h, the reaction mixture was cooled to 0 °C, at which point a solution of 4′-(trifluoromethyl)-4-biphenylamine (0.43 mmol), pyridine (0.86 mmol), and acetonitrile (1.0 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 1 min and then allowed to warm to room temperature slowly. After 15 h, the reaction mixture was diluted with water (60 mL) and brine (10 mL) and extracted with (3 × 50 mL) ethyl acetate. The combined organic layers were dried over sodium sulfate and then concentrated in vacuo. Purification by silica gel chromatography (20-50% ethyl acetate/hexanes) afforded the title compound as a white solid (57%). ^{1}H NMR (DMSO-d_6): δ 9.75 (s, 1H), 7.85 (d, 2H, $J = 8.1$ Hz), 7.77 (d, 2H, $J = 8.3$ Hz), 7.68 (d, 2H, $J = 8.6$ Hz), 7.28 (d, 2H, $J = 8.6$ Hz), 7.22 (s, 2H). MS *m*/*z* 317.2 $[M + H]^+$. Anal. ($C_{13}H_{11}F_3N_2O_2S$) C, H, N.

***N*-Methyl-*N*′-[4′-(trifluoromethyl)-4-biphenylyl]urea (21).** To a solution of 4′-(trifluoromethyl)-4-biphenylamine (0.421 mmol) in dichloromethane (2 mL) was added methyl isocyanate (0.505 mmol). After stirring 18 h at room temperature, the reaction mixture was quenched with water (2 mL), stirred for 1 h, and concentrated in vacuo. The residue was purified by reverse phase HPLC to afford the title product as a white solid (22%). ^{1}H NMR (DMSO-d_6): δ 8.70 (s, 1H), 7.85 (d, 2H, $J = 8.3$ Hz), 7.77 (d, 2H, $J = 8.4$ Hz), 7.64 (d, 2H, $J = 8.7$ Hz), 7.54 (d, 2H, $J = 8.7$ Hz), 6.08 (q, 1H, $J = 4.4$ Hz), 2.66 (d, 3H, $J = 4.3$ Hz). MS *m*/*z* 295.2 $[M + H]^+$. Anal. ($C_{15}H_{13}F_3N_2O$) C, H, N.

***N*,*N*-Dimethyl-*N*′-[4′-(trifluoromethyl)-4-biphenylyl]urea (22).** Sodium hydride (7.00 mmol of a 60% suspension in mineral oil) was added to an ice-cooled, stirred solution of 4-bromophenyl isocyanate (3.53 mmol) and guanidine hydrochloride (31.4 mmol) in DMF (30 mL) under nitrogen. After stirring at room temperature for 18 h, the mixture was poured into water (300 mL) and acidified with 1 M aq hydrochloric acid. The solid was filtered, washed with water and ether, then dried to give *N*-[amino(imino)methyl]-*N*′-(4-bromophenyl)urea as a white solid (47%) contaminated with a small amount (13%) of bisacylated guanidine. ^{1}H NMR (DMSO-d_6) δ 10.41 (br s, 1H), 9.09 (br s, 1H), 7.54 (d, 2H, $J = 9.1$ Hz), 7.50 (d, 2H, $J = 9.1$ Hz). MS *m*/*z* 257 $[M + H]^+$. A stirred mixture of the urea (1.00 mmol), 4-(trifluoromethyl)phenylboronic acid (2.00 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.05 mmol) in DMF (3 mL) and 2 M aq potassium carbonate (3 mL) was heated at 100 °C under argon for 5 h, then cooled and diluted with 1 M aq hydrochloric acid (45 mL). The precipitate was filtered, washed with water and dried. The crude product was purified by silica gel chromatography (50−70% ethyl acetate/hexanes) to give the title compound as a solid (55%). ^{1}H NMR (DMSO-d_6) δ 8.46 (s, 1H), 7.87 (d, 2H, $J = 8.2$ Hz), 7.77 (d, 2H, $J = 8.3$ Hz), 7.64 (m, 4H), 2.96 (s, 6H). MS *m*/*z* 309 $[M + H]^+$. Analytical HPLC 99.7% purity, $t_R = 7.51$.

***N*,*N*-Dimethyl-*N*′-[4′-(trifluoromethyl)-4-biphenylyl]sulfamide (23).** To a solution of 4′-(trifluoromethyl)-4-biphenylamine (0.42 mmol) in dichloromethane (2.0 mL) was sequentially added triethylamine (1.26 mmol) and dimethylaminesulfamoyl chloride (0.63 mmol). The reaction mixture was stirred at room temperature for 18 h and then concentrated in vacuo. Purification by reverse phase HPLC afforded the title product as a white solid (35%). ^{1}H NMR (DMSO-d_6) δ 10.15 (s, 1H), 7.88 (d, 2H, $J = 8.2$ Hz), 7.79 (d, 2H, $J = 8.4$ Hz), 7.71 (d, 2H, $J = 8.7$ Hz), 7.51 (d, 2H, $J = 8.7$ Hz), 2.73 (s, 6H). MS *m*/*z* 345.2 $[M + H]^+$. Anal. ($C_{15}H_{15}F_3N_2O_2S$) C, H, N.

***N*-Methyl-*N*-[4′-(trifluoromethyl)-4-biphenylyl]sulfamide (24).** To a solution of *N*-[4′-(trifluoromethyl)-4-biphenylyl]sulfamide (0.36 mmol) in DMF (2.0 mL) was added sodium hydride (0.38 mmol). The reaction mixture was stirred at room temperature for 10 min and was treated with methyl iodide (0.47 mmol). After 18 h, the reaction mixture was diluted with water (10 mL) and brine (10 mL) and extracted with (3 × 20 mL) ethyl acetate. The combined organic layers were dried over sodium sulfate and then concentrated in vacuo. Purification of the residue by reverse phase HPLC afforded the title compound as a white solid (57%). ^{1}H NMR (DMSO-d_6): δ 7.90 (d, 2H, $J = 7.6$ Hz), 7.82 (d, 2H, $J = 7.6$ Hz), 7.76 (d, 2H, $J = 7.1$ Hz), 7.48 (d, 2H, $J = 7.2$ Hz), 7.12 (s, 2H), 3.16 (s, 3H). MS *m*/*z* 331.2 $[M + H]^+$. Anal. ($C_{14}H_{13}F_3N_2O_2S$) C, H, N.

1-[4′-(Trifluoromethyl)-4-biphenylyl]methanesulfonamide (25). To a solution of 1-bromo-4-(bromomethyl)benzene (4.00 mmol) in acetonitrile (12.0 mL) was added a solution of sodium sulfite (4.80 mmol) in water (8.0 mL).[46] The biphasic mixture was heated at reflux (87 °C oil bath) for 16 h and then concentrated in vacuo to remove the acetonitrile. The resultant solid was filtered and rinsed with water (2 × 5 mL) and dichloromethane (3 × 5 mL). The sodium salt of (4-bromophenyl)methanesulfonic acid was dried to constant weight to afford a white powder (54%). ^{1}H NMR (DMSO-d_6): δ 7.44 (d, 2H, $J = 8.4$ Hz), 7.25 (d, 2H, $J = 8.4$ Hz), 3.66 (s, 2H). MS *m*/*z* 248.8 $[M - H]^-$. This intermediate (0.73 mmol) was slurried in DMF (5.0 mL), cooled to 0 °C, and treated dropwise with thionyl chloride (2.71 mmol). After 15 min, the cooling bath was removed and the reaction mixture was warmed to room temperature. After 2 h, the reaction mixture was carefully poured into ice cold water (75 mL), and the organics were extracted with (3 × 50 mL) ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated in vacuo. The crude residue was carefully treated with aq ammonium hydroxide solution (4.0 mL). After 24 h at room temperature, a white precipitate was filtered and rinsed with water. Concentration in vacuo provided the crude

intermediate 1-(4-bromophenyl)methanesulfonamide as a white powder (56%). ^{1}H NMR (DMSO-d_6): δ 7.59 (d, 2H, J = 8.3 Hz), 7.32 (d, 2H, J = 8.3 Hz), 6.87 (s, 2H), 4.26 (s, 2H). MS m/z 169.2 $[M - SO_2NH_2]^+$. The title compound was prepared as a white glassy solid from this intermediate following Method A, with purification by silica gel chromatography (25−50% ethyl acetate/hexanes) and then recrystallization from hot isopropanol (43%). ^{1}H NMR (DMSO-d_6): δ 7.92 (d, 2H, J = 8.4 Hz), 7.84 (d, 2H, J = 8.3 Hz), 7.77 (d, 2H, J = 8.1 Hz), 7.51 (d, 2H, J = 8.3 Hz), 6.91 (s, 2H), 4.34 (s, 2H). MS m/z 235.2 $[M - SO_2NH_2]^+$; 428.2 $[M + CF_3CO_2^-]^-$. Anal. ($C_{14}H_{12}F_3NO_2S$) C, H, N.

***N*-Methyl-1-[4′-(trifluoromethyl)-4-biphenylyl]methanesulfonamide (26).** The sodium salt of (4-bromophenyl)methanesulfonic acid (0.37 mmol) was slurried in DMF (2.0 mL), cooled to 0 °C, and treated dropwise with thionyl chloride (1.35 mmol). After 10 min, the cooling bath was removed and the reaction mixture was warmed to room temperature. After 2 h, the reaction mixture was carefully poured into ice cold water (65 mL), and the organics were extracted with (3 × 50 mL) ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated in vacuo. The crude residue was carefully treated with a 40% solution of methylamine in water (2.0 mL). After 3.5 h at room temperature, the reaction mixture was heated at 40 °C for 16 h. A white precipitate was filtered and rinsed with water. Concentration in vacuo provided the crude intermediate 1-(4-bromophenyl)-*N*-methylmethanesulfonamide as a white powder (33%). ^{1}H NMR (DMSO-d_6): δ 7.59 (d, 2H, J = 8.3 Hz), 7.33 (d, 2H, J = 8.3 Hz), 6.95 (q, 1H, J = 4.6 Hz), 4.34 (s, 2H), 2.57 (d, 3H, J = 4.6 Hz). MS m/z 169.2 $[M - SO_2NHCH_3]^+$. The title compound was prepared as a white powder from this intermediate following Method A (31%). ^{1}H NMR (DMSO-d_6): δ 7.92 (d, 2H, J = 8.3 Hz), 7.83 (d, 2H, J = 8.3 Hz), 7.77 (d, 2H, J = 8.4 Hz), 7.51 (d, 2H, J = 8.4 Hz), 6.99 (br s, 1H), 4.41 (s, 2H), 2.61 (br s, 3H). MS m/z 235.2 $[M - SO_2NHCH_3]^+$; 442.2 $[M + CF_3CO_2^-]^-$. Analytical HPLC 99.6% purity, t_R = 7.56.

4′-(Trifluoromethyl)-4-biphenylyl Sulfamate (27). The title compound was prepared as a white powder from 4′-(trifluoromethyl)-4-biphenylol following Method D (25%). ^{1}H NMR (DMSO-d_6): δ 8.05 (br s, 2H), 7.90 (d, 2H, J = 8.3 Hz), 7.84 (d, 4H, J = 8.6 Hz), 7.41 (d, 2H, J = 8.6 Hz). MS m/z 318.2 $[M + H]^+$. Analytical HPLC 94.26% purity, t_R = 7.55.

4′-(Trifluoromethyl)-4-biphenylyl Carbamate (28). The intermediate 4′-(trifluoromethyl)-4-biphenylol was prepared as a white solid following Method A using 4-bromophenol (71%). ^{1}H NMR ($CDCl_3$): δ 7.63 (m, 4H), 7.48 (d, 2H, J = 8.7 Hz), 6.92 (d, 2H, J = 8.7 Hz), 4.82 (s, 1H). The title compound was prepared as a white powder (88%) from this phenol following Method C with purification by silica gel chromatography (20−45% ethyl acetate/hexanes). ^{1}H NMR (DMSO-d_6): δ 7.89 (d, 2H, J = 8.3 Hz), 7.82 (d, 2H, J = 8.4 Hz), 7.74 (d, 2H, J = 8.5 Hz), 7.25 (m, 3H), 6.99 (br s, 1H). MS m/z 282.2 $[M + H]^+$. Anal. ($C_{14}H_{10}F_3NO_2$) C, H, N.

***N*-[3-Fluoro-4′-(trifluoromethyl)-4-biphenylyl]urea (29).** The intermediate [3-fluoro-4′-(trifluoromethyl)-4-biphenylyl]amine was prepared following Method A using 4-bromo-2-fluoroaniline (93%). ^{1}H NMR (DMSO-d_6): δ 7.80 (d, 2H, J = 8.3 Hz), 7.71 (d, 2H, J = 8.4 Hz), 7.46 (dd, 1H, J = 12.9, 2.0 Hz), 7.34 (dd, 1H, J = 8.3, 2.0 Hz), 6.85 (m, 1H), 5.48 (s, 2H). MS m/z 256.0 $[M + H]^+$. The title compound was prepared as a white solid from this aniline following Method C (56%). ^{1}H NMR (DMSO-d_6): δ 8.52 (d, 1H, J = 2.0 Hz), 8.30 (app t, 1H, J = 8.6 Hz), 7.90 (d, 2H, J = 8.2 Hz), 7.79 (d, 2H, J = 8.3 Hz), 7.66 (dd, 1H, J = 12.9, 2.0 Hz), 7.53 (dd, 1H, J = 8.6, 1.8 Hz), 6.29 (s, 2H). MS m/z 299.2 $[M + H]^+$. Analytical HPLC 95.4% purity, t_R = 6.84.

***N*-[3-Fluoro-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (30).** The title compound was prepared from [3-fluoro-4′-(trifluoromethyl)-4-biphenylyl]amine following Method D (30%). ^{1}H NMR (DMSO-d_6): δ 9.42 (s, 1H), 7.92 (d, 2H, J = 8.3 Hz), 7.80 (d, 2H, J = 8.3 Hz), 7.67 (d, 1H, J = 11.1 Hz), 7.62-7.56 (m, 2H), 7.25 (s, 2H). MS m/z 335.2 $[M + H]^+$. Anal. ($C_{13}H_{10}F_4N_2O_2S$) H, N, C: calcd, 46.71; found, 45.91. Analytical HPLC 99.5% purity, t_R = 7.00.

***N*-[3-Cyano-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (31).** The intermediate 4-amino-4′-(trifluoromethyl)-3-biphenylcarbonitrile was prepared following Method A using 2-amino-5-bromobenzonitrile (87%). ^{1}H NMR (DMSO-d_6): δ 7.86-7.83 (m, 3H), 7.77-7.73 (m, 3H), 6.90 (d, 1H, J = 8.8 Hz), 6.38 (s, 2H). MS m/z 263.2 $[M + H]^+$. The title compound was prepared from this aniline following Method D (45%). ^{1}H NMR (DMSO-d_6): δ 9.77 (s, 1H), 8.21 (d, 1H, J = 1.5 Hz), 8.08 (d, 1H, J = 8.3 Hz), 7.97 (d, 2H, J = 8.3 Hz), 7.83 (d, 2H, J = 8.3 Hz), 7.71 (d, 1H, J = 8.3 Hz), 7.38 (s, 2H). MS m/z 342.0 $[M + H]^+$, 364.2 $[M + Na]^+$. Anal. ($C_{14}H_{10}F_3N_3O_2S$) C, H, N.

***N*-[3-Methyl-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (32).** Crude intermediate 3-methyl-4′-(trifluoromethyl)-4-biphenylamine was prepared following Method A using 4-bromo-2-methylaniline. MS m/z 252.5 $[M + H]^+$. The title compound was prepared as a white solid from this aniline following Method D (25%). ^{1}H NMR (DMSO-d_6): δ 8.68 (s, 1H), 7.88 (d, 2H, J = 8.4 Hz), 7.80 (d, 2H, J = 8.4 Hz), 7.59 (s, 1H), 7.56 (dd, 1H, J = 8.3, 2.2 Hz), 7.50 (d, 1H, J = 8.3 Hz), 7.02 (s, 2H), 2.37 (s, 3H). MS m/z 331.2 $[M + H]^+$. Anal. ($C_{14}H_{13}F_3N_2O_2S$) C, H, N.

***N*-[3-(Methoxy)-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (33).** 3-Methoxy-4-nitro-4′-(trifluoromethyl)biphenyl was prepared following Method A using 5-chloro-2-nitroanisole (42%). ^{1}H NMR (DMSO-d_6): δ 8.03 (dd, 3H, J = 8.2, 4.9 Hz), 7.89 (d, 2H, J = 8.3 Hz), 7.64 (d, 1H, J = 1.8 Hz), 7.48 (dd, 1H, J = 8.3, 1.8 Hz), 4.05 (s, 3H). MS m/z 298.2 $[M + H]^+$. To a solution of this biphenyl (1.96 mmol) in acetic acid (10 mL) was added zinc dust (13.7 mmol). The reaction mixure was stirred at room temperature for 2 h, filtered through celite, and the filter pad was washed with acetic acid (5 mL) and ethanol (5 mL). The filtrate was concentrated in vacuo, and the residue was taken up in ethyl acetate (15 mL) and washed with saturated aqueous sodium carbonate solution (10 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo to give the intermediate [3-(methoxy)-4′-(trifluoromethyl)-4-biphenylyl]amine as an off-white solid (quantitative). ^{1}H NMR (DMSO-d_6): δ 7.82 (d, 2H, J = 8.1 Hz), 7.71 (d, 2H, J = 8.3 Hz), 7.17 (d, 1H, J = 2.0 Hz), 7.12 (dd, 1H, J = 8.1, 1.8 Hz), 6.73 (d, 1H, J = 8.1 Hz), 5.06 (s, 2H), 3.87 (s, 3H). MS m/z 268.0 $[M + H]^+$. The title compound was prepared as a white solid from this aniline following Method D (19%). ^{1}H NMR (DMSO-d_6): δ 8.20 (s, 1H), 7.92 (d, 2H, J = 8.2 Hz), 7.80 (d, 2H, J = 8.2 Hz), 7.50 (d, 1H, J = 8.3 Hz), 7.35 (s, 1H), 7.31 (d, 1H, J = 8.3 Hz), 7.18 (s, 2H), 3.92 (s, 3H). MS m/z 267.2 $[M - SO_2NH_2]^+$. Anal. ($C_{14}H_{13}F_3N_2O_3S$) C, H, N.

***N*-[3-Hydroxy-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (34).** To a solution of [3-(methoxy)-4′-(trifluoromethyl)-4-biphenylyl]amine (0.68 mmol) in 1-methyl-2-pyrrolidinone (0.34 mL) was added potassium carbonate (0.034 mmol) and thiophenol (0.68 mmol). The reaction mixture was irradiated in the microwave at 220 °C for 15 min. The reaction mixture was diluted with ethyl acetate (10 mL) and concentrated in vacuo. Purification of the residue by silica gel chromatography (20−65% ethyl acetate/hexanes) afforded the intermediate 4-amino-4′-(trifluoromethyl)-3-biphenylol as a brown solid (82%). ^{1}H NMR (DMSO-d_6): δ 9.25 (s, 1H), 7.70 (s, 4H), 7.03-6.98 (m, 2H), 6.68 (d, 1H, J = 8.0 Hz), 4.86 (s, 2H). MS m/z 254.2 $[M + H]^+$. To a solution of the phenol (0.395 mmol) in pyridine (2.0 mL) was added sulfamide (0.435 mmol). The reaction mixture was heated at reflux for 2 h. The reaction mixture was then cooled to room temperature and concentrated in vacuo. Purification of the residue by reverse phase HPLC afforded the title compound as a light brown solid (22%). ^{1}H NMR (DMSO-d_6): δ 9.97 (s, 1H), 7.99 (s, 1H), 7.79 (s, 4H), 7.43 (d, 1H, J = 8.1 Hz), 7.19-7.10 (m, 4H). MS m/z 333.2 $[M + H]^+$. Analytical HPLC 96.9% purity, t_R = 6.30.

***N*-[3-Amino-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (35).** 3-Nitro-4′-(trifluoromethyl)-4-biphenylamine was prepared following Method A with 4-bromo-2-nitroaniline (79%). ^{1}H NMR

(DMSO-d_6): δ 8.31 (d, 1H, J = 2.3 Hz), 7.89-7.84 (m, 3H), 7.80-7.75 (m, 2H), 7.65 (s, 2H), 7.15 (d, 1H, J = 8.8 Hz). MS m/z 283.2 $[M + H]^+$. The intermediate *N*-[3-nitro-4′-(trifluoromethyl)-4-biphenylyl]sulfamide was prepared from the aniline following Method D (69%). ^{1}H NMR (DMSO-d_6): δ 9.62 (s, 1H), 8.44 (d, 1H, J = 2.3 Hz), 8.19 (dd, 1H, J = 8.8, 2.3 Hz), 8.00 (d, 2H, J = 8.1 Hz), 7.86 (dd, 3H, J = 8.5, 2.6 Hz), 7.76 (s, 2H). MS m/z 362.0 $[M + H]^+$. A solution of the sulfamide (0.73 mmol) in ethanol (10 mL) was reduced in a Parr shaker at 50 psi H_2 with platinum-(IV) oxide (0.01 mmol). After 15 min, the reaction mixture was filtered through celite and concentrated in vacuo. Purification by reverse phase HPLC afforded the title product as a white solid (81%). ^{1}H NMR (DMSO-d_6): δ 8.40 (s, 1H), 7.80-7.74 (m, 4H), 7.24 (d, 1H, J = 8.1 Hz), 7.03 (d, 1H, J = 2.0 Hz), 6.91 (s, 2H), 6.88 (dd, 1H, J = 8.2, 2.2 Hz), 5.21 (s, 2H). MS m/z 332.2 $[M + H]^+$. Anal. ($C_{13}H_{12}F_3N_3O_2S$) C, H, N.

***N*-[3-(Methylamino)-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (36).** *N*-Methyl-4-nitro-4′-(trifluoromethyl)-3-biphenylamine was prepared following Method A using 5-chloro-*N*-methyl-2-nitroaniline (68%). ^{1}H NMR (DMSO-d_6): δ 8.31 (d, 1H, J = 4.8 Hz), 8.19 (d, 1H, J = 9.1 Hz), 8.01 (d, 2H, J = 8.1 Hz), 7.88 (d, 2H, J = 8.3 Hz), 7.22 (d, 1H, J = 1.5 Hz), 7.03 (dd, 1H, J = 8.8, 1.8 Hz), 3.07 (d, 3H, J = 5.0 Hz). MS m/z 297.2 $[M + H]^+$. To a solution of this biphenyl (1.09 mmol) in acetic acid (7 mL) was added zinc dust (7.63 mmol). The reaction mixure was stirred at room temperature for 2 h, filtered through celite, and the filter pad was washed with acetic acid (10 mL) and ethanol (10 mL). The filtrate was concentrated in vacuo, and the residue was taken up in ethyl acetate (10 mL) and washed with saturated aqueous sodium bicarbonate solution (10 mL). The organic layer was dried over magnesium sulfate and concentrated in vacuo to give the intermediate 4-amino-4′-(trifluoromethyl)-3-biphenylyl]methylamine as an off-white solid (77%). ^{1}H NMR (DMSO-d_6): δ 7.77 (d, 2H, J = 8.3 Hz), 7.69 (d, 2H, J = 8.4 Hz), 6.85 (dd, 1H, J = 7.8, 2.0 Hz), 6.71 (d, 1H, J = 1.8 Hz), 6.63 (d, 1H, J = 8.1 Hz), 4.82 (s, 2H), 4.79 (d, 1H, J = 4.8 Hz), 2.80 (d, 3H, J = 5.0 Hz). MS m/z 267.0 $[M + H]^+$. A solution of the aniline (0.38 mmol) and sulfamide (1.9 mmol) in 1,4-dioxane (1.0 mL) was irradiated in the microwave at 150 °C for 10 min. The reaction mixture was concentrated in vacuo. Purification of the residue by reverse phase HPLC afforded the title compound as a pink solid (5%). ^{1}H NMR (DMSO-d_6): δ 8.40 (s, 1H), 7.86 (d, 2H, J = 8.3 Hz), 7.78 (d, 2H, J = 8.6 Hz), 7.26 (d, 1H, J = 8.1 Hz), 6.93-6.85 (m, 3H), 6.82 (d, 1H, J = 1.5 Hz), 5.41 (br s, 1H), 2.80 (s, 3H). MS m/z 346.2 $[M + H]^+$. Analytical HPLC 97.3% purity, t_R = 6.18.

***N*-[3-Chloro-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (37).** Crude intermediate 3-chloro-4′-(trifluoromethyl)-4-biphenylamine was prepared following Method A using 4-bromo-2-chloroaniline. MS m/z 272.0 $[M + H]^+$. The title compound was prepared as a white solid from this aniline following Method D with purification by reverse phase HPLC (35%). ^{1}H NMR (DMSO-d_6): δ 9.96 (s, 1H), 7.82 (d, 2H, J = 8.1 Hz), 7.65 (d, 2H, J = 8.1 Hz), 7.40-7.36 (m, 4H), 7.20 (dd, 1H, J = 8.4, 2.2 Hz). MS m/z 351.2 $[M + H]^+$. Anal. ($C_{13}H_{10}ClF_3N_2O_2S$) C, H, N.

***N*-[2-Fluoro-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (38).** Crude intermediate 2-fluoro-4′-(trifluoromethyl)-4-biphenylamine was prepared following Method A using 4-bromo-3-fluoroaniline. MS m/z 256.2 $[M + H]^+$. The title compound was prepared as a white solid from this aniline following Method D with purification by reverse phase HPLC (35%). ^{1}H NMR (DMSO-d_6): δ 10.04 (s, 1H), 7.82 (d, 2H, J = 8.3 Hz), 7.75 (d, 2H, J = 8.1 Hz), 7.53 (t, 1H, J = 8.7 Hz), 7.38 (s, 2H), 7.12 (dd, 1H, J = 13.2, 2.2 Hz), 7.06 (dd, 1H, J = 8.5, 2.2 Hz). MS m/z 335.2 $[M + H]^+$. Anal. ($C_{13}H_{10}F_4N_2O_2S$) C, H, N.

***N*-[3,5-Difluoro-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (39).** Crude intermediate 3,5-difluoro-4′-(trifluoromethyl)-4-biphenylamine was prepared following Method A using 4-bromo-2,6-difluoroaniline. MS m/z 274.0 $[M + H]^+$. The title compound was prepared as a white solid from this aniline following Method D with purification by reverse phase HPLC (16%). ^{1}H NMR (DMSO-d_6): δ 8.95 (s, 1H), 7.99 (d, 2H, J = 8.3 Hz), 7.85 (d, 2H, J = 8.3 Hz), 7.62 (d, 2H, J = 8.8 Hz), 7.07 (s, 2H). MS m/z 353.2 $[M + H]^+$. Analytical HPLC 95.4% purity, t_R = 8.80.

***N*-(4′-Chloro-4-biphenylyl)methanesulfonamide (40).** The title compound was prepared as a yellow solid (82%) from *N*-(4-bromophenyl)methanesulfonamide[47] and (4-chlorophenyl)boronic acid following Method A. ^{1}H NMR (DMSO-d_6): δ 9.91 (s, 1H), 7.67 (m, 4H), 7.51 (d, 2H, J = 8.5 Hz), 7.30 (d, 2H, J = 8.6 Hz), 3.03 (s, 3H). MS m/z 282.0 $[M + H]^+$. Anal. ($C_{13}H_{12}ClNO_2S$) H, N. Analytical HPLC 95.4% purity, t_R = 7.36.

***N*-[4′-(2,2,2-Trifluoroethyl)-4-biphenylyl]methanesulfonamide (41).** To a solution of 4-bromobenzyl bromide (20 mmol) in 1-methyl-2-pyrrolidinone (25 mL) was added copper(I) iodide (5.2 mmol) and methyl 2,2-difluoro-2-(fluorosulfonyl)acetate (39 mmol). The reaction mixture was purged with nitrogen and heated at 80 °C for 24 h. After cooling, the reaction mixture was diluted with water, and the organics were extracted with hexanes, filtered through a pad of celite, and concentrated in vacuo. Purification of the residue by silica gel chromatography (hexanes) gave the intermediate 4-(2,2,2-trifluoroethyl)bromobenzene as a white solid (61%, contained ~15% 4-bromobenzyl bromide). ^{1}H NMR ($CDCl_3$): δ 7.51 (d, 2H, J = 8.3 Hz), 7.19 (d, 2H, J = 8.3 Hz), 3.35 (q, 2H, J = 10.7 Hz). Intermediate 4-[4′-(2,2,2-trifluoroethyl)-4-biphenylyl]amine was prepared as a yellow solid from this bromide and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline following Method A (100%). ^{1}H NMR ($CDCl_3$): δ 7.54 (d, 2H, J = 8.4 Hz), 7.37 (d, 2H, J = 8.5 Hz), 7.34 (d, 2H, J = 8.5 Hz), 6.64 (d, 2H, J = 8.4 Hz), 5.26 (s, 2H), 3.63 (q, 2H, J = 11.6 Hz). MS m/z 252.0 $[M + H]^+$. The title compound was prepared from 4-[4′-(2,2,2-trifluoroethyl)-4-biphenylyl]amine following Method B. Purification of the residue by silica gel chromatography (35% ethyl acetate/hexanes), trituration with hexanes, filtration, and concentration in vacuo afforded the title product as a white solid (94%). ^{1}H NMR ($CDCl_3$): δ 9.88 (s, 1H), 7.67 (d, 2H, J = 8.6 Hz), 7.65 (d, 2H, J = 8.1 Hz), 7.44 (d, 2H, J = 8.1 Hz), 7.30 (d, 2H, J = 8.6 Hz), 3.68 (m, 2H, J = 11.6 Hz), 3.03 (s, 3H). MS m/z 330.2 $[M + H]^+$. Anal. ($C_{15}H_{14}F_3NO_2S$) C, H, N.

***N*-[4′-(Trifluoromethoxy)-4-biphenylyl]methanesulfonamide (42).** The title compound was prepared from *N*-(4-bromophenyl)methanesulfonamide[47] and 4-(trifluoromethoxy)phenyl boronic acid following Method A. Purification by reverse phase HPLC provided the title product as a white powder (25%). ^{1}H NMR (DMSO-d_6): δ 10.10 (s, 1H), 8.16 (d, 2H, J = 8.6 Hz), 8.13 (d, 2H, J = 8.6 Hz), 7.85 (d, 2H, J = 8.4 Hz), 7.36 (d, 2H, J = 8.3 Hz), 3.08 (s, 3H). MS m/z 332.4 $[M + H]^+$. Anal. ($C_{14}H_{12}F_3NO_3S$) C, H, N.

***N*-{4′-[(Trifluoromethyl)sulfonyl]-4-biphenylyl}methanesulfonamide (43).** *N*-[4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl]methanesulfonamide was prepared in 30% yield with 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline following Method B with purification by flash chromatography (50−100% ethyl acetate/hexanes). ^{1}H NMR (DMSO-d_6): δ 10.05 (s, 1H), 7.62 (d, 2H, J = 8.5 Hz), 7.20 (d, 2H, J = 8.5 Hz), 3.03 (s, 3H), 1.28 (s, 12H). MS m/z 298.2 $[M + H]^+$. The title compound was prepared from this boronate and 1-bromo-4-[(trifluoromethyl)-sulfonyl]benzene following Method A with purification by reverse phase HPLC (61%). ^{1}H NMR (DMSO-d_6): δ 10.13 (s, 1H), 8.18 (d, 2H, J = 8.6 Hz), 8.12 (d, 2H, J = 8.7 Hz), 7.85 (d, 2H, J = 8.7 Hz), 7.36 (d, 2H, J = 8.7 Hz), 3.09 (s, 3H). MS m/z 380.2 $[M + H]^+$. Anal. ($C_{14}H_{12}F_3NO_4S_2$) C, H, N.

***N*-4-Biphenylylurea (44).** The title compound was prepared as a white powder from 4-biphenylamine following Method C (27%). ^{1}H NMR (DMSO-d_6): δ 8.64 (s, 1H), 7.62 (d, 2H, J = 8.4 Hz), 7.55 (d, 2H, J = 8.8 Hz), 7.49 (d, 2H, J = 8.8 Hz), 7.43 (t, 2H, J = 7.7 Hz), 7.30 (m, 1H), 5.90 (s, 2H). MS m/z 213.2 $[M + H]^+$. Analytical HPLC 95.0% purity, t_R = 5.23.

***N*-[3′-(Trifluoromethyl)-4-biphenylyl]urea (45).** The intermediate 3′-(trifluoromethyl)-4-biphenylamine was prepared as a brown solid following Method A using 3-trifluoromethylphenyl boronic acid (48%). ^{1}H NMR ($CDCl_3$): δ 7.76 (s, 1H), 7.69 (m, 1H), 7.50 (m, 2H), 7.41 (d, 2H, J = 8.6 Hz), 6.76 (d, 2H, J = 8.5 Hz), 3.60 (br s, 2H). MS m/z 238.0 $[M + H]^+$. The title compound was

prepared as a tan solid from this aniline following Method C (55%). ^{1}H NMR (DMSO-d_6): δ 8.70 (s, 1H), 7.95 (m, 1H), 7.91 (s, 1H), 7.68-7.63 (m, 4H), 7.54 (d, 2H, J = 8.6 Hz), 5.92 (s, 2H). MS m/z 281.0 $[M + H]^+$. Anal. ($C_{14}H_{11}F_3N_2O$) C, H, N.

***N*-(4′-Methyl-4-biphenylyl)urea (46).** The intermediate 4′-methyl-4-biphenylamine was prepared following Method A using 4-methylphenyl boronic acid (37%). ^{1}H NMR ($CDCl_3$): δ 7.42-7.35 (m, 4H), 7.18 (d, 2H, J = 8.3 Hz), 6.73 (d, 2H, J = 8.6 Hz), 3.67 (br s, 2H), 2.35 (s, 3H). MS m/z 184.0 $[M + H]^+$. The title compound was prepared as a tan solid from this aniline following Method C (59%). ^{1}H NMR (DMSO-d_6): δ 8.60 (s, 1H), 7.52-7.46 (m, 6H), 7.23 (d, 2H, J = 8.1 Hz), 5.86 (s, 2H), 2.33 (s, 3H). MS m/z 227.2 $[M + H]^+$. Analytical HPLC 99.3%, t_R = 5.89.

***N*-(4′-Isopropyl-4-biphenylyl)urea (47).** The intermediate 4′-isopropyl-4-biphenylamine was prepared following Method A using 4-isopropylphenyl boronic acid (38%). ^{1}H NMR ($CDCl_3$): δ 7.45 (d, 2H, J = 8.3 Hz), 7.38 (d, 2H, J = 8.6 Hz), 7.24 (d, 2H, J = 8.1 Hz), 6.73 (d, 2H, J = 8.6 Hz), 3.68 (br s, 2H), 2.92 (septet, 1H, J = 6.9 Hz), 1.26 (d, 6H, J = 6.9 Hz). MS m/z 212.2 $[M + H]^+$. The title compound was prepared as a tan solid from this aniline following Method C (44%). ^{1}H NMR (DMSO-d_6): δ 8.59 (s, 1H), 7.53-7.45 (m, 6H), 7.29 (d, 2H, J = 8.3 Hz), 5.86 (s, 2H), 2.91 (septet, 1H, J = 6.9 Hz), 1.23 (d, 6H, J = 6.8 Hz). MS m/z 255.2 $[M + H]^+$. Anal. ($C_{16}H_{18}N_2O$) H, N, C: calcd, 75.56; found, 74.82. Analytical HPLC 94.6% purity, t_R = 7.09.

***N*-(4′-*t*-Butyl-4-biphenylyl)urea (48).** The intermediate 4′-*t*-butyl-4-biphenylamine was prepared following Method A using 4-*t*-butylphenyl boronic acid (51%). ^{1}H NMR ($CDCl_3$): δ 7.46 (d, 2H, J = 8.5 Hz), 7.40 (m, 4H), 6.73 (d, 2H, J = 8.5 Hz), 3.69 (br s, 2H), 1.33 (s, 9H). MS m/z 226.2 $[M + H]^+$. The title compound was prepared as a tan solid from this aniline following Method C (55%). ^{1}H NMR (DMSO-d_6): δ 8.60 (s, 1H), 7.55-7.43 (m, 8H), 5.86 (s, 2H), 1.31 (s, 9H). MS m/z 269.2 $[M + H]^+$. Analytical HPLC 99.1% purity, t_R = 7.52.

General Method for Suzuki Cross-Coupling. Method E: *N*-(4′-Bromo-3,3′-difluoro-4-biphenylyl)urea (49). The intermediate *N*-(4-bromo-2-fluorophenyl)urea was prepared in 70% yield following Method C using 4-bromo-2-fluoroaniline and was purified by recrystallization from hot ethyl acetate/methanol (5:1). ^{1}H NMR (DMSO-d_6): δ 8.45 (d, 1H, J = 2.0 Hz), 8.13 (app t, 1H, J = 9.0 Hz), 7.50 (dd, 1H, J = 11.1, 2.3 Hz), 7.28 (m, 1H), 6.25 (s, 2H). MS m/z 233.0 $[M + H]^+$. A solution of the urea (0.54 mmol), 4-bromo-3-fluorobenzeneboronic acid (0.64 mmol), and dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium(II)-dichloromethane adduct (0.02 mmol) in DMF (2 mL) and 2 M aq sodium carbonate (1.61 mmol) was irradiated in the microwave at 110 °C for 7 min. The resulting dark solution was treated with sodium sulfate (~500 mg) and decolorizing charcoal (~50 mg) and filtered through a syringe fitted with a nylon filter disc. The filtrate was purified by reverse phase HPLC and the subsequent precipitated product was collected, filtered, washed with water, and dried in vacuo to afford the title product as a tan solid (10%). MS m/z 328.9 $[M + H]^+$. Analytical HPLC 100.0% purity, t_R = 6.82.[48]

***N*-{4′-[(Trifluoromethyl)thio]-4-biphenyl}urea (50).** The intermediate 4′-[(trifluoromethyl)thio]-4-biphenylamine was prepared as a tan solid following Method A using 4-bromophenyl trifluoromethyl sulfide and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline with purification by reverse phase HPLC (45%). ^{1}H NMR (DMSO-d_6): δ 8.07-7.99 (m, 4H), 7.61 (d, 2H, J = 8.6 Hz), 6.71 (d, 2H, J = 8.3 Hz), 5.74 (br s, 2H). MS m/z 270.2 $[M + H]^+$. The title compound was prepared as a white solid from this aniline following Method C (55%). ^{1}H NMR (DMSO-d_6): δ 8.62 (s, 1H), 7.71-7.65 (m, 4H), 7.53 (d, 2H, J = 8.8 Hz), 7.44 (d, 2H, J = 8.6 Hz), 5.85 (s, 2H). MS m/z 313.0 $[M + H]^+$. Analytical HPLC 94.3% purity, t_R = 7.26.

***N*-{4′-[(Trifluoromethyl)sulfonyl]-4-biphenyl}urea (51).** The intermediate 4′-[(trifluoromethyl)sulfonyl]-4-biphenylamine was prepared as a yellow solid following Method A using 4-chlorophenyl trifluoromethyl sulfone and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (85%). ^{1}H NMR (DMSO-d_6): δ 8.06-7.97 (m, 4H), 7.59 (d, 2H, J = 8.8 Hz), 6.69 (d, 2H, J = 8.6 Hz), 5.71 (s, 2H). MS m/z 302.2 $[M + H]^+$. The title compound was prepared as a white solid from this aniline following Method C (35%). ^{1}H NMR (DMSO-d_6): δ 8.83 (s, 1H), 8.19-8.02 (m, 4H), 7.75 (d, 2H, J = 8.8 Hz), 7.59 (d, 2H, J = 8.6 Hz), 6.00 (s, 2H). MS m/z 345.2 $[M + H]^+$. Anal. ($C_{14}H_{11}F_3N_2O_3S$) C, H, N.

***N*-[4′-(Ethylsulfonyl)-3-fluoro-4-biphenylyl]urea (52).** The title compound was prepared as a white solid following Method E with 4-(ethanesulfonyl)benzeneboronic acid (36%). ^{1}H NMR (DMSO-d_6): δ 8.55 (d, 1H, J = 2.5 Hz), 8.31 (app t, 1H, J = 8.8 Hz), 7.96 (d, 2H, J = 8.9 Hz), 7.91 (d, 2H, J = 8.6 Hz), 7.69 (dd, 1H, J = 12.9, 2.0 Hz), 7.56 (dd, 1H, J = 8.5, 1.8 Hz), 6.30 (s, 2H), 3.33 (q, 2H, J = 7.3 Hz), 1.12 (t, 3H, J = 7.3 Hz). MS m/z 323.0 $[M + H]^+$. Anal. ($C_{15}H_{15}FN_2O_3S$) C, H, N.

***N*-[4′-(Trifluoroacetyl)-4-biphenylyl]urea (53).** The intermediate 1-(4′-amino-4-biphenylyl)-2,2,2-trifluoroethanone was prepared as a bright yellow solid following Method A with 2,2,2-trifluoro-1-(4-iodophenyl)ethanone[49] and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline at 80 °C for 1.5 h (50%). ^{1}H NMR (DMSO-d_6): δ 8.03 (d, 2H, J = 7.6 Hz), 7.87 (d, 2H, J = 8.8 Hz), 7.57 (d, 2H, J = 8.8 Hz), 6.66 (d, 2H, J = 8.4 Hz), 5.65 (s, 2H). MS m/z 266.0 $[M + H]^+$. The title compound was prepared as a yellow solid from this aniline following Method C with purification by silica gel chromatography (ethyl acetate) followed by recrystallization from acetonitrile (13%). TLC R_f = 0.33 (EtOAc). ^{1}H NMR (DMSO-d_6): δ 8.80 (s, 1H), 8.10 (d, 2H, J = 8.0 Hz), 7.96 (d, 2H, J = 8.8 Hz), 7.73 (d, 2H, J = 8.8 Hz), 7.57 (d, 2H, J = 8.8 Hz), 5.98 (s, 2H). MS m/z 341.2 $[M + H + H_2O]^+$. Anal. ($C_{15}H_{11}F_3N_2O_2$) C, H, N.

***N*-[3′-Fluoro-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (54).** The intermediate 3′-fluoro-4′-(trifluoromethyl)-4-biphenylamine was prepared as a white powder following Method A using 4-bromo-2-fluoro-1-(trifluoromethyl)benzene and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (86%). ^{1}H NMR (DMSO-d_6): δ 7.69 (m, 2H), 7.58 (d, 1H, J = 8.3 Hz), 7.52 (d, 2H, J = 8.6 Hz), 6.68 (d, 2H, J = 8.3 Hz), 5.75 (s, 2H). MS m/z 256.2 $[M + H]^+$. The title compound was prepared as a white solid from this aniline following Method D with purification by reverse phase HPLC (24%). ^{1}H NMR (DMSO-d_6): δ 9.83 (s, 1H), 7.82 (m, 2H), 7.76-7.69 (m, 3H), 7.27 (m, 4H). MS m/z 335.2 $[M + H]^+$. Anal. ($C_{13}H_{10}F_4N_2O_2S$) H, N. Analytical HPLC 95.2% purity, t_R = 7.01.

***N*-[3′-Nitro-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (55).** The intermediate 3′-nitro-4′-(trifluoromethyl)-4-biphenylamine was prepared following Method A using 4-bromo-2-nitro-1-(trifluoromethyl)benzene and 4-aminophenyl boronic acid (78%). ^{1}H NMR (DMSO-d_6): δ 8.27 (d, 1H, J = 1.6 Hz), 8.06 (dd, 1H, J = 8.6, 1.2 Hz), 7.94 (d, 1H, J = 8.4 Hz), 7.59 (d, 2H, J = 8.6 Hz), 6.68 (d, 2H, J = 8.6 Hz), 5.66 (br s, 2H). MS m/z 283.2 $[M + H]^+$. The title compound was prepared from this aniline following Method D (74%). ^{1}H NMR (DMSO-d_6): δ 9.90 (s, 1H), 8.41 (d, 1H, J = 1.5 Hz), 8.19 (d, 1H, J = 8.3 Hz), 8.06 (d, 1H, J = 8.6 Hz), 7.81 (d, 2H, J = 8.6 Hz), 7.31 (s, 2H), 7.30 (d, 2H, J = 8.6 Hz). MS m/z 362.2 $[M + H]^+$. Analytical HPLC 96.4% purity, t_R = 6.75.

***N*-[3′-Amino-4′-(trifluoromethyl)-4-biphenylyl]sulfamide (56).** To a stirred solution of *N*-[3′-nitro-4′-(trifluoromethyl)-4-biphenylyl]-sulfamide (0.78 mmol) in acetic acid (10 mL) was added zinc powder (5.4 mmol). The suspension was stirred at room temperature for 18 h and then filtered through a pad of celite. The filter pad was rinsed with acetic acid, and the filtrate was concentrated in vacuo. The residue was taken up in ethyl acetate, washed sequentially with 1 N aqueous sodium carbonate solution and brine, dried over sodium sulfate, and concentrated in vacuo. Purification by flash chromatography on silica gel (40−60% ethyl acetate/dichloromethane), trituration with hexanes, and drying in vacuo provided the title compound as a white solid (85%). ^{1}H NMR (DMSO-d_6): δ 9.70 (s, 1H), 7.52 (d, 2H, J = 8.6 Hz), 7.37 (d, 1H, J = 8.3 Hz), 7.24 (d, 2H, J = 8.6 Hz), 7.19 (br s, 2H), 7.07 (s, 1H), 6.87 (d, 1H, J = 8.3 Hz), 5.63 (s, 2H). MS m/z 332.2 $[M + H]^+$. Anal. ($C_{13}H_{12}F_3N_3O_2S$) C, H, N.

General Method for Suzuki Cross-Coupling. Method F: 4-(1,3-Benzodioxol-5-yl)aniline. A solution of 3,4-(methylenedioxy)phenylboronic acid (5.14mmol), 4-iodoaniline (3.42 mmol), dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium(II)-dichloromethane adduct (0.10 mmol), and cesium carbonate (17.1 mmol) in DMF (10 mL) and water (3 mL) was heated at 100 °C for 20 h. The reaction mixture was cooled, poured into brine (50 mL), and extracted with (3 × 50 mL) ethyl acetate. The combined organic layers were dried over magnesium sulfate and decolorizing charcoal, filtered through celite, and concentrated in vacuo. Purification of the residue by reverse phase HPLC and neutralization of the collected fractions afforded the title product as an off-white solid (60%). MS *m*/*z* 214.1 $[M + H]^+$.

***N*-[4-(1,3-Benzodioxol-5-yl)phenyl]sulfamide (57).** The title compound was prepared from 4-(1,3-benzodioxol-5-yl)aniline following Method D (39%). ^{1}H NMR (DMSO-d_6): δ 9.57 (s, 1H), 7.52 (d, 2H, J = 8.1 Hz), 7.23-7.17 (m, 3H), 7.14 (s, 2H), 7.09 (dd, 1H, J = 8.1, 1.8 Hz), 6.97 (d, 1H, J = 8.1 Hz), 6.05 (s, 2H). MS *m*/*z* 293.2 $[M + H]^+$. Anal. ($C_{13}H_{12}N_2O_4S$) H, N, C: calcd 53.42; found 52.79. Analytical HPLC 98.0% purity, t_R = 5.32.

***N*-[4-(2,2-Difluoro-1,3-benzodioxol-5-yl)phenyl]sulfamide (58).** The intermediate 4-(2,2-difluoro-1,3-benzodioxol-5-yl)aniline was prepared following Method F using 5-bromo-2,2-difluoro-1,3-benzodioxole, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-aniline, and tetrakis(triphenylphosphine)palladium(0) (50%). ^{1}H NMR (DMSO-d_6): δ 7.57 (d, 1H, J = 1.5 Hz), 7.41-7.28 (m, 4H), 6.65-6.58 (m, 2H), 5.30 (s, 2H). MS *m*/*z* 250.2 $[M + H]^+$. The title compound was prepared from this aniline following Method D (31%). ^{1}H NMR (DMSO-d_6): δ 9.67 (s, 1H), 7.69 (s, 1H), 7.58 (d, 2H, J = 8.6 Hz), 7.45 (s, 2H), 7.23 (d, 2H, J = 8.8 Hz), 7.18 (s, 2H). MS *m*/*z* 329.3 $[M + H]^+$. Anal. ($C_{13}H_{10}F_2N_2O_4S$) C, H, N.

***N*-[4-(2,2-Dimethyl-1,3-benzodioxol-5-yl)phenyl]sulfamide (59).** A solution of 3,4-*O*-isopropylidinebromobenzene[50] (2.62 mmol), [4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]amine (3.14 mmol), and dichloro[1,1′-bis(diphenylphosphino)ferrocene]palladium-(II)-dichloromethane adduct (0.26 mmol) in acetonitrile (2.6 mL) and 2 M aqueous sodium carbonate solution (2.62 mL) was irradiated in the microwave at 150 °C for 8 min. The reaction mixture was filtered through celite, concentrated in vacuo, and purified by reverse phase HPLC to afford the intermediate 4-(2,2-dimethyl-1,3-benzodioxol-5-yl)aniline (32%). MS *m*/*z* 242.0 $[M + H]^+$.

Approximately half of the 4-(2,2-dimethyl-1,3-benzodioxol-5-yl)aniline was dissolved in dioxane (1 mL) and was treated with sulfamide (3.3 mmol). The reaction mixture was irradiated in the microwave at 150 °C for 10 min. The reaction mixture was concentrated in vacuo and the residue was purified by reverse phase HPLC. Precipitation of the product from water and filtration with a hexanes wash provided the title compound (36 mg). ^{1}H NMR (DMSO-d_6): δ 9.55 (s, 1H), 7.49 (d, 2H, J = 8.6 Hz), 7.19 (d, 2H, J = 8.6 Hz), 7.13-7.09 (m, 3H), 7.04 (m, 1H), 6.87 (d, 1H, J = 8.1 Hz), 1.66 (s, 6H). MS *m*/*z* 321.2 $[M + H]^+$. Anal. ($C_{15}H_{16}N_2O_4S$) C, H, N.

***N*-[4-(2,3-Dihydro-1,4-benzodioxin-6-yl)phenyl]sulfamide (60).** A solution of 6-bromo-2,3-dihydro-1,4-benzodioxin (1.20 mmol), [4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]amine (1.32 mmol), cesium carbonate (2.08 mmol), and dichloro[1,1′-bis-(diphenylphosphino)ferrocene]palladium(II)-dichloromethane adduct (0.02 mmol) in DMF (6 mL) was irradiated in the microwave at 110 °C for 10 min. The reaction was cooled, diluted with diethyl ether, and extracted with aqueous sodium bicarbonate solution. The organic layer was concentrated to give 0.223 g of crude [4-(2,3-dihydro-1,4-benzodioxin-6-yl)phenyl]amine (83%). MS *m*/*z* 228.0 $[M + H]^+$. The title compound was prepared from this aniline following Method D with purification by reverse phase HPLC (25%). ^{1}H NMR (DMSO-d_6): δ 9.55 (s, 1H), 7.54-7.43 (m, 2H), 7.22-7.16 (m, 2H), 7.15-7.04 (m, 4H), 6.90 (d, 1H, J = 8.3 Hz), 4.27 (s, 4H). MS *m*/*z* 307.2 $[M + H]^+$. Anal. ($C_{14}H_{14}N_2O_4S$) C, H, N.

***N*-[4-(2,2,3,3-Tetrafluoro-2,3-dihydro-1,4-benzodioxin-6-yl)-phenyl]sulfamide (61).** A solution of 5-bromo-2,2,3,3-tetrafluoro-1,4-benzodioxane (1.20 mmol), [4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]amine (1.32 mmol), cesium carbonate (2.08 mmol), and dichloro[1,1′-bis(diphenylphosphino)ferrocene]-palladium(II)-dichloromethane adduct (0.02 mmol) in DMF (6 mL) was irradiated in the microwave at 110 °C for 10 min. The reaction mixture was diluted with diethyl ether and washed with saturated aqueous sodium bicarbonate solution. The organic layer was concentrated to give 0.275 g of crude [4-(2,2,3,3-tetrafluoro-2,3-dihydro-1,4-benzodioxin-6-yl)phenyl]amine. MS *m*/*z* 300.0 $[M + H]^+$. The title compound was prepared from this aniline following Method D with purification by reverse phase HPLC (23%). ^{1}H NMR (DMSO-d_6): δ 9.73 (s, 1H), 7.75 (s, 1H), 7.69-7.58 (m, 3H), 7.53 (m, 1H), 7.31-7.15 (m, 4H). MS *m*/*z* 379.2 $[M + H]^+$. Anal. ($C_{14}H_{10}F_4N_2O_4S$) C, H, N.

***N*-[4-(2,2,4,4-Tetrafluoro-4*H*-1,3-benzodioxin-6-yl)phenyl]sulfamide (62).** The intermediate [4-(2,2,4,4-tetrafluoro-4*H*-1,3-benzodioxin-6-yl)phenyl]amine was prepared following Method A using 6-bromo-2,2,4,4-tetrafluoro-4*H*-1,3-benzodioxin and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (75%). ^{1}H NMR (DMSO-d_6): δ 8.07 (s, 1H), 8.02 (d, 1H, J = 8.8 Hz), 7.61 (d, 2H, J = 8.8 Hz), 7.52 (d, 1H, J = 8.8 Hz), 6.93 (d, 2H, J = 8.8 Hz). MS *m*/*z* 300.2 $[M + H]^+$. The title compound was prepared from this aniline following Method D with purification by reverse phase HPLC (12%). ^{1}H NMR (DMSO-d_6): δ 9.74 (s, 1H), 8.13 (s, 1H), 8.07 (d, 1H, J = 8.4 Hz), 7.70 (d, 2H, J = 8.8 Hz), 7.56 (d, 1H, J = 8.8 Hz), 7.26 (d, 2H, J = 9.2 Hz), 7.23 (s, 2H). MS *m*/*z* 379.2 $[M + H]^+$. Anal. ($C_{14}H_{10}F_4N_2O_4S$) C, H, N.

Biology. Biochemical Characterization. The KSP ATPase assay was performed utilizing 15 μM ATP and 25 nM KSP motor domain as previously described.[33,36] Unless indicated, data are reported as the average of two or more experiments (each run in duplicate). Statistical limits for the data are reported as the standard deviation of two or more experiments or the standard error within one experiment. Mode of inhibition studies and inhibition constant calculations were conducted on all key analogs as described previously (selected results presented).[36]

Inhibition of Cellular Proliferation. SKOV3, Colo205, and HCT116 cell lines were obtained from the ATCC. The HCT116 D130V derivative was created by continuous exposure to a quinazolinone KSP inhibitor.[28] Cells were grown in plastic tissue culture flasks containing RPMI 1640 medium supplemented with 10% fetal calf serum (v/v) and were maintained at 37 °C in a humidified atmosphere containing 5% carbon dioxide. Test compounds in DMSO stock solutions were serially diluted in DMSO, diluted with growth medium, and then diluted into the cell assay plate with a final DMSO concentration of ≤0.2%. Cells were plated in duplicate 96-well plates at 1000 cells/well and were allowed to adhere for 24 h. Test compounds were added to the wells. All plates contained a DMSO control and wells with no cells to subtract nonspecific background. Cell assay plates were incubated at 37 °C for 72 h and then harvested and read using the CellTiter-Glo (CTG) Luminescent Cell Viability Assay (Promega #G7571) on a Wallac EnVision 2100 Multichannel Reader to determine the number of viable cells. The extent of growth inhibition was determined by comparison of the number of cells remaining after 72 h exposure to the growth of cells in control wells that had been treated with vehicle alone (0.2% DMSO). Dose−response curves were analyzed with XLFit or Grafit and the value from the cells treated with DMSO alone was considered 100%. Unless indicated, data are reported as the average of two or more experiments (each run in duplicate). Statistical limits for the data are reported as the standard deviation of two or more experiments or the standard error within one experiment. Cellular phenotype resulting from inhibitor treatment was assessed using a Cytometrix automated cell imaging protocol.

In Vivo Mouse Studies. All in vivo studies were performed in accordance with protocols approved by the GSK Institutional Animal Care and Use Committee and met or exceeded the standards

of the American Association for the Accreditation of Laboratory Animal Care (AAALAC), the United States Department of Health and Human Services, and all local and federal animal welfare laws.

For xenograft models, Colo205 and HCT116 D130V tumors were grown subcutaneously in female athymic nude mice (CD-1 Nu/Nu mice). Compounds were formulated in 2% Cremophor EL, 2% *N,N*-dimethylacetamide, and 96% acidified water (pH 5.0) and administered intraperitoneally on a q4dx3 schedule (three doses each separated by 4 days). Tumors were measured twice weekly and the tumor volume was calculated using the following formula [(length × width^2) × 0.5]. Data are presented as the median of five animals. The median tumor volume at the start of the efficacy studies was 100−200 mm^3. Delays in tumor growth were determined by comparison of the times when compound-treated mice and placebo-treated mice each reached a median tumor volume of 1000 mm^3. Objective responses were defined as follows: complete regression (CR), three consecutive measurements of 13.5 mm^3 or less; partial regression (PR), three consecutive measurements of ≤50% of initial volume.

For pharmacokinetic studies, compound was administered as a single intraperitoneal dose to female athymic nude mice as described above. At each timepoint (5, 10, 60, 120, 240, 360, and 1440 min), three mice were bled by cardiac puncture under terminal anesthesia. Plasma samples were analyzed by HPLC/MS/MS after protein precipitation with acetonitrile. Data was analyzed using WinNonlin version 3.2 with noncompartmental analysis.

Acknowledgment. We gratefully acknowledge Stanley J. Schmidt, Michael G. Darcy, Jian Jin, Deana F. Wang, Yonghui Wang, and Dongchuan Shi for early SAR contributions. We also thank Jim Bulgarelli, Eric Manning, and Ganesh Moorthy for analytical mouse PK support, Yan Lee (Cytokinetics) for initial biochemical characterization of compound **5**, Hong Lu for technical assistance, and Chao Han for helpful discussions.

Supporting Information Available: Full elemental analyses on noted compounds and statistical limits for biological data in Tables 1 and 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM070435Y