Journal of Medicinal Chemistry

Article

pubs.acs.org/jmc

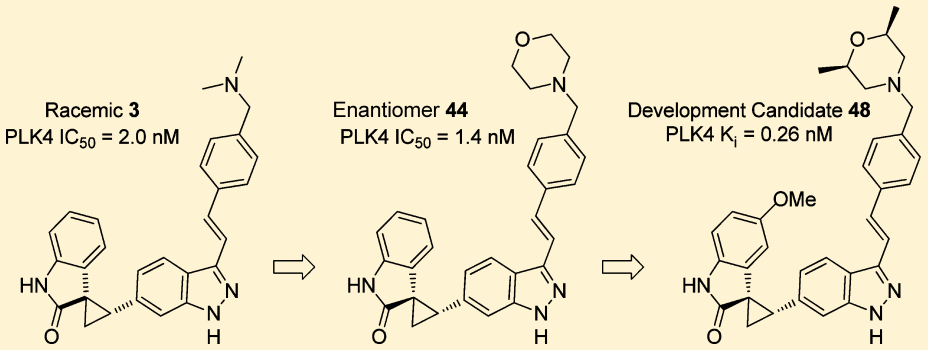
The Discovery of Polo-Like Kinase 4 Inhibitors: Identification of (1*R*,2*S*)-2-(3-((*E*)-4-(((*cis*)-2,6-Dimethylmorpholino)methyl)styryl)-1*H*-indazol-6-yl)-5′-methoxyspiro[cyclopropane-1,3′-indolin]-2′-one (CFI-400945) as a Potent, Orally Active Antitumor Agent

Peter B. Sampson,[†] Yong Liu,[†] Bryan Forrest,[†] Graham Cumming,[‡] Sze-Wan Li,[†] Narendra Kumar Patel,[†] Louise Edwards,[†] Radoslaw Laufer,[†] Miklos Feher,[†] Fuqiang Ban,[†] Donald E. Awrey,[†] Guodong Mao,[†] Olga Plotnikova,[†] Richard Hodgson,[†] Irina Beletskaya,[†] Jacqueline M. Mason,[†] Xunyi Luo,[†] Vincent Nadeem,[†] Xin Wei,[†] Reza Kiarash,[†] Brian Madeira,[†] Ping Huang,[†] Tak W. Mak,[†] Guohua Pan,[†] and Henry W. Pauls*[,†]

[†]Campbell Family Institute for Breast Cancer Research, University Health Network, TMDT East Tower, MaRS Centre, 101 College Street, Toronto, Ontario MG5 1L7, Canada

[‡]Celtic Catalysts, 1-03 Nova Centre, Belfield, Dublin 4, Ireland

Supporting Information



ABSTRACT: Previous publications from our laboratory have introduced novel inhibitors of Polo-like kinase 4 (PLK4), a mitotic kinase identified as a potential target for cancer therapy. The search for potent and selective PLK4 inhibitors yielded (*E*)-3-((1*H*-indazol-6-yl)methylene)indolin-2-ones, which were superseded by the bioisosteric 2-(1*H*-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-ones, e.g., **3**. The later scaffold confers improved drug-like properties and incorporates two stereogenic centers. This work reports the discovery of a novel one-pot double S_N2 displacement reaction for the stereoselective installation of the desired asymmetric centers and confirms the stereochemistry of the most potent stereoisomer, e.g., **44**. Subsequent work keys on the optimization of the oral exposure of nanomolar PLK4 inhibitors with potent cancer cell growth inhibitory activity. A short list of compounds with superior potency and pharmacokinetic properties in rodents and dogs was studied in mouse models of tumor growth. We conclude with the identification of compound **48** (designated CFI-400945) as a novel clinical candidate for cancer therapy.

INTRODUCTION

The Polo-like kinase (PLK) family of serine/threonine kinases plays a critical role in the regulation of mitosis.[1] The family derives its name from the Polo-box domains (PBD) incorporated in the proteins, which are distinct and separate from the kinase (ATP binding) pocket.[2] The PBDs bind phosphorylated peptide sequences, and this interaction plays a role in the localization of Polo-like kinases to subcellular structures. Of the five members described to date, PLK1, 2, 3, 4, and 5, PLK1 is the most studied member. Multiple PLK1 inhibitors have been described, and several have been taken into the clinic.[3] By contrast, PLK4 has, as yet, received little attention. This enzyme is structurally the most distinct member of the family.[4] Unlike PLK1, 2, and 3, PLK4 has one Polo box only and an active site with high homology to the Aurora kinases. PLK4 has a restricted tissue distribution, being present only in proliferating tissues.

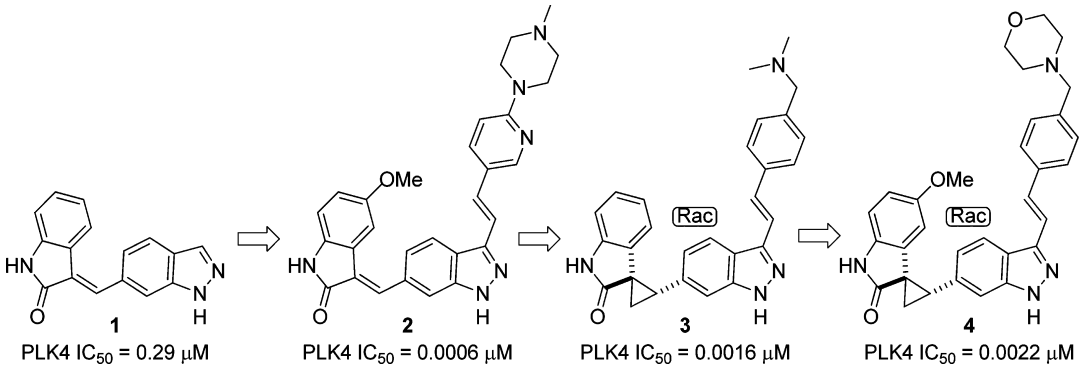
PLK4 localizes to the centrosomes and is a critical regulator of centriole duplication.[5−7] Deregulation of PLK4 results in loss of centrosome numerical integrity and leads to chromosome instability (CIN), a characteristic observed in many types of cancers.[8−10] It has been shown that PLK4 is up-regulated in breast cancer, specifically in the basal-like subtype, and that

Special Issue: New Frontiers in Kinases

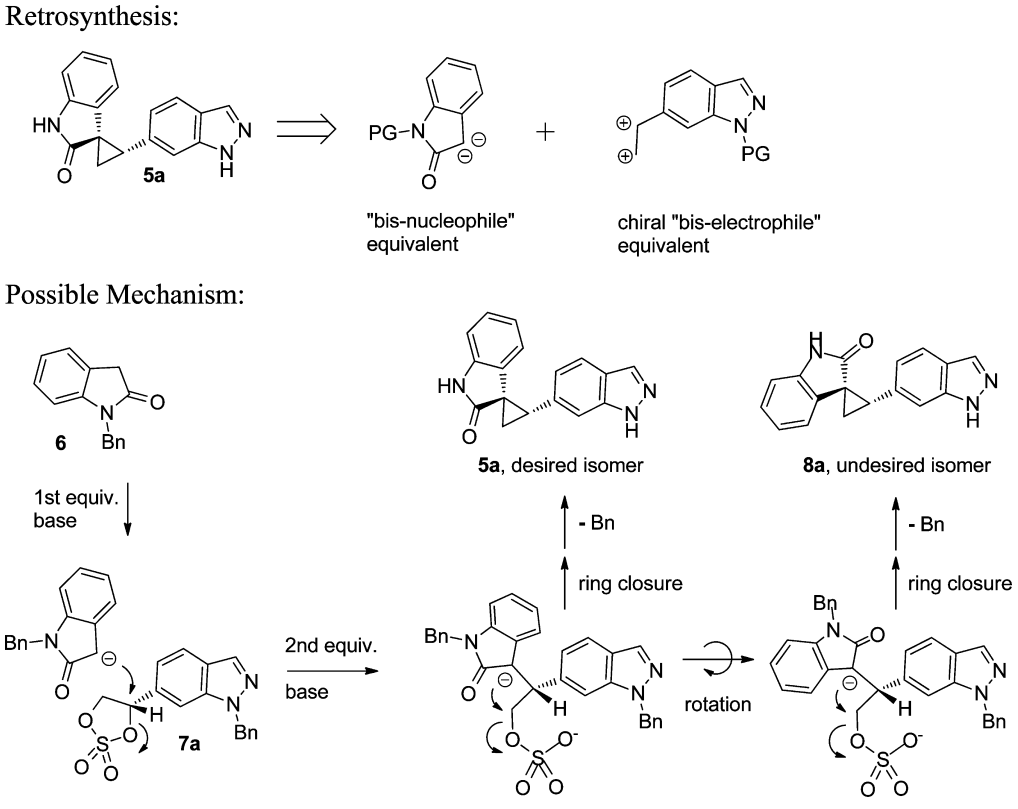
Received: April 3, 2014

   dx.doi.org/10.1021/jm5005336 | *J. Med. Chem.* XXXX, XXX, XXX−XXX

Scheme 1. Systematic Optimization of PLK4 Inhibitors



Scheme 2. Preparation of Chiral Indazolyl-spiro[cyclopropane-indolinones] via a Double S_N2 Displacement



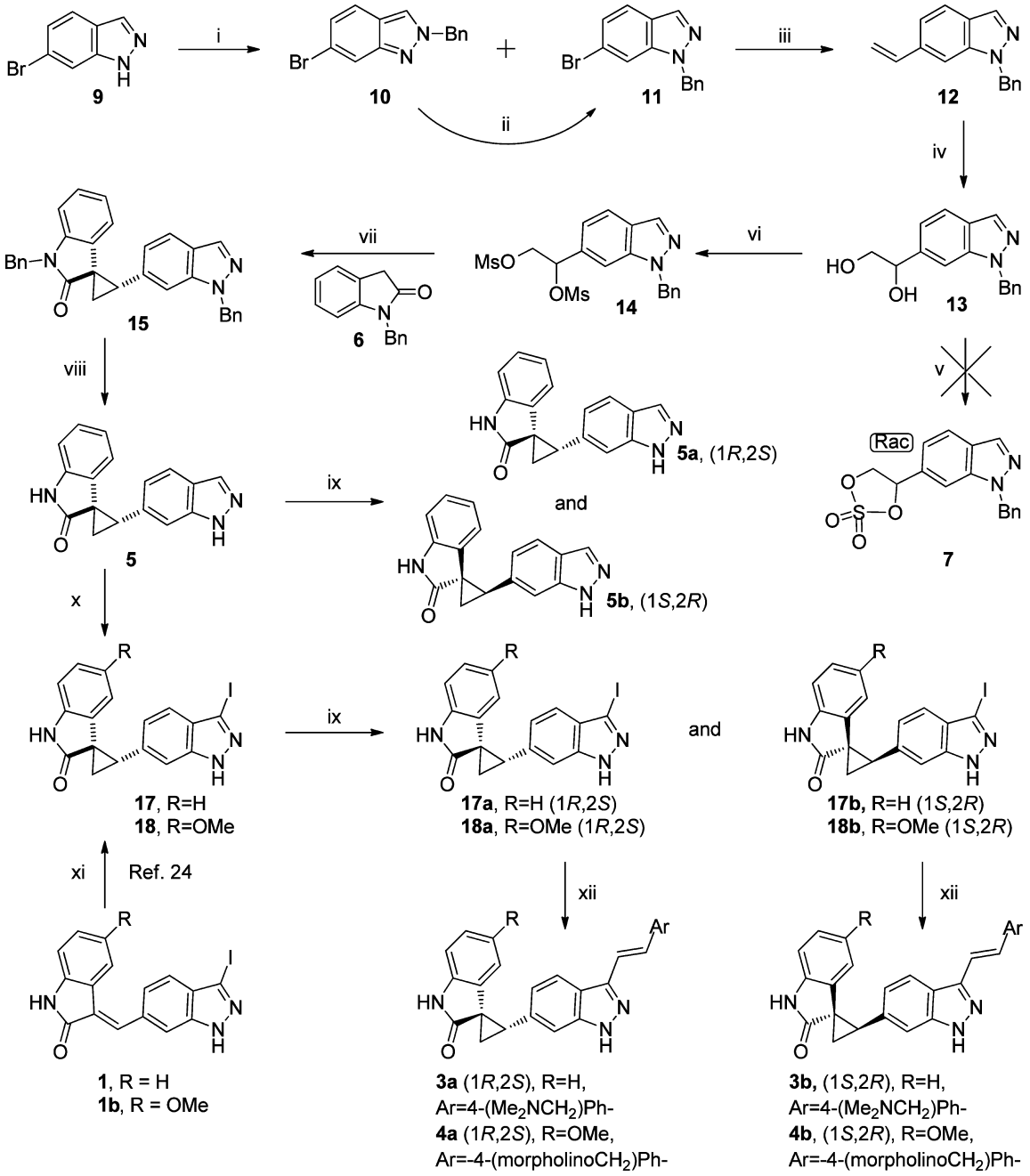
high PLK4 levels are associated with poor patient outcomes.[11−13] We have shown that treatment of breast cancer cells with PLK4 RNAi prevents centriole duplication and results in cell death while normal breast cells are unaffected.[14,15] Consistent with these observations, RNAi-mediated depletion of PLK4 in breast cancer xenografts causes growth suppression. These findings identified PLK4 as a potential target for anticancer therapy and caused us to initiate a drug discovery effort.[16,17]

The most common strategy for the inhibition of kinases is binding to the ATP site; potent inhibitors result but the potential for cross reactivity with other kinases is well documented.[18−21] An alternative approach to inhibition is possible for the Polo-like kinase family. A priori, binding to the polo-box domain could yield specific inhibitors and overcome drug resistance often associated with ATP competitive kinase inhibitors.[22] The PBD is unique for the family of PLKs and is essential for PLK functions and so is a potentially useful site for the development of potent inhibitors for clinical uses. Unfortunately, the known ligands for PBD sites are generally rather large phosphorylated peptide sequences.[23] These are unsuitable starting points for lead generation, and thus our approach was dictated by the availability of drug-like hits for the inhibition of PLK4, which turned out to be ATP competitive hinge binders.[16,17]

Our previous publication on PLK4 inhibition described the discovery of a novel series of ATP competitive kinase inhibitors, namely (*E*)-3-((1*H*-indazol-6-yl)methylene)indolin-2-ones **1** (Scheme 1). Subsequent optimization of PLK4 activity by several orders of magnitude was accomplished by substituting the indolinone ring and appending an aryl vinyl moiety on the indazole, giving compound **2**.[17] The preceding work[24] describes the bioisosteric replacement of the alkene linker with a cyclopropane ring to yield (2-(1*H*-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-ones, for example, compound **3**. This change resulted in a substantial improvement in drug-like properties

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

Scheme 3. Synthesis and Chiral Separation of Racemic (1*R*,2*S*)-2-(1*H*-Indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-ones[a]



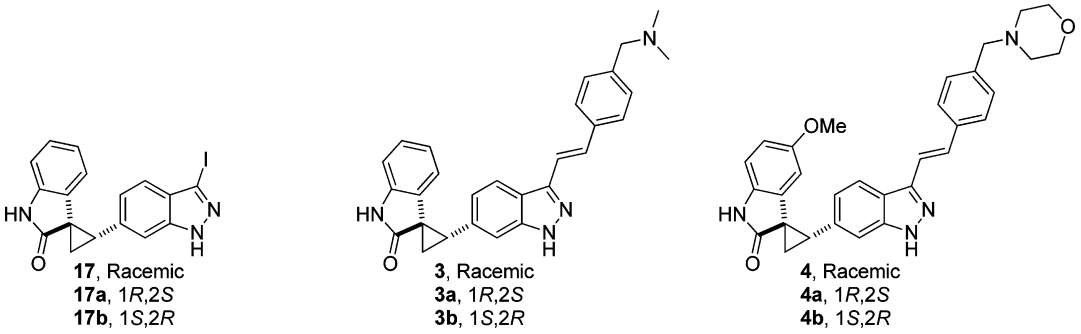
[a]Reagents and conditions: (i) BnCl, DMSO, *t*-BuOK, 4 h; (ii) BnCl, 150 °C, 8 h; (iii) 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane, $Pd(PPh_3)_2Cl_2$ (2 mol %), K_2CO_3, DME/H_2O, 80 °C, 7 h; (iv) $K_2OsO_4{\cdot}2H_2O$ (0.5 mol %), NMO (1.1 equiv), citric acid (2 equiv), *t*-BuOH/H_2O, rt 16 h then 50 °C; (v) SO_2Cl_2, TEA, CH_2Cl_2, rt; (vi) MsCl, TEA, CH_2Cl_2, 0 °C; (vii) NaH (60% in oil), THF; (viii) DMSO, *t*-BuOK, THF, O_2; (ix) chiral preparative SFC; (x) I_2, K_2CO_3; (xi) $Me_3S(O)I$, NaH, DMF, rt; (xii) ArCH═CHBpin, 2:1 PhMe/EtOH, 1 M aq Na_2CO_3, $Pd(PPh_3)_4$ (5 mol %), microwave 120 °C, 2 h or 1 M aq Na_2CO_3, LiCl, $Pd(PPh_3)_4$ (2.5 mol %), dioxane, reflux, 18 h. Relative and absolute stereochemistry indicated by unwedged (bold and hashed) and wedged (bold and hashed) lines, respectively.

such as oral bioavailability, which were further refined by morphing the dimethyl amino moiety to a morpholine-containing compound **4**.

Besides the documented beneficial effects,[24] the change from an alkene to cyclopropane linker had stereochemical consequences: the new series has two stereogenic centers. Fortuitously, the chemistry employed to install the cyclopropane ring yielded the more active diastereomer as the major product, albeit as a racemic mixture of enantiomers. It was anticipated that only one of these enantiomers was responsible for the potent PLK4 activity observed. The X-ray structure presented in the preceding paper showed that the active enantiomer was likely to have the 1*R*,2*S* stereochemistry.[24] In fact, our modeling studies indicated that this same enantiomer would be favored by up to 2 log units of binding energy. In addition, because development of racemic mixtures is undesirable, a chiral route to our final compounds was required.

dx.doi.org/10.1021/jm5005336 | *J. Med. Chem.* XXXX, XXX, XXX−XXX

Table 1. Effect of Spirocyclopropane Stereochemistry on PLK4 and Cell Growth Inhibition



	17	17a	17b	3	3a	3b	4	4a	4b
stereochemistry	racemic	1*R*,2*S*	1*S*,2*R*	racemic	1*R*,2*S*	1*S*,2*R*	racemic	1*R*,2*S*	1*S*,2*R*
PLK4 IC_{50} (μM)	0.65	0.29	~30	0.002	0.00062	0.24	0.0022	0.0009	0.12
MDA-MB-468 GI_{50} (μM)	ND	ND	ND	<0.01	<0.01	0.7	<0.01	<0.01	0.14

A number of strategies for the preparation of asymmetric spirocyclopropanes were contemplated. The most direct approach is to apply some chiral version of the ylide chemistry (Corey–Chaykovsky reaction) used for the racemic synthesis.[24] However, cyclopropanations using chiral sulfoxonium ylides do not provide high levels of asymmetric induction and generally fail when applied to complex systems.[25] Chiral sulfonium ylides have shown greater success,[26] but even simple reagents, such as dimethylsulfonium methylide, failed to react with the (*E*)-3-((1*H*-indazol-6-yl)methylene)indolin-2-one substrates. Our approach to the preparation of asymmetric cyclopropanes was based on the double S_N2 displacement of a chiral bis-electrophile with bis-nucleophile (Scheme 2). A simple racemic version of this reaction has been described by Marti and Carreira[27] using a cyclic sulfate as the bis-electrophile.[28] It was envisaged that the desired reaction would proceed via an inversion of stereochemistry at the benzylic carbon, i.e., reaction of the anion of indolinone **6** with the asymmetric cyclic sulfate **7a**. Hydrophobic collapse and/or a favorable π-stacking interaction during the subsequent ring closure induce formation of the desired isomer at the quaternary center of the cyclopropane. This sets the stereochemistry as 1*R*,2*S*; removal of the benzyl protecting group gives compound **5a**, the desired isomer. Conversely, rotation of the indolinone, ring closure and benzyl deprotection would yield the undesired diastereomer **8a**.

In this work, we verify the configuration of the most active stereoisomer and describe the synthetic chemistry developed to obtain the same, stereoselectively, with high enantiomeric purity. This breakthrough, preliminarily disclosed,[29] set the stage for further optimization of the series, resulting in the identification of compound **48**, a potent, orally active PLK4 inhibitor as an antitumor agent for clinical development.

RESULTS AND DISCUSSION

Chemical Synthesis. Our preliminary studies were performed with racemic substrate in order to test the feasibility of the double displacement chemistry. Scheme 3 outlines the preparation of the requisite starting material. Treatment of commercially available bromoindazole **9** with benzyl chloride and potassium *tert*-butoxide generated a 1:3 mixture of *N*2 and *N*1-benzylated indazoles, **10** and **11**, respectively. The mixture was isomerized exclusively to the *N*1 benzylated indazole **11** by heating to 150 °C in excess benzyl chloride. Suzuki–Miyaura coupling with vinyl boronic acid pinacol ester gave vinyl indazole **12**, in good yield, which was smoothly converted to racemic diol **13**. Unfortunately, reaction of **13** with thionyl chloride failed to yield the cyclic sulfate **7**. In contrast, treatment of diol **13** with methanesulfonyl chloride readily generated racemic **14**, an alternate version of the required bis-electrophile. To test the feasibility of the key reaction, **14** and oxindole **6** were treated with sodium hydride in THF. We observed rapid conversion to one major product, consistent with the racemate **15**. Similar to the Marti and Carreira result,[27] the reaction had proceeded with high relative stereoselectivity to yield the desired diastereomer (94% de). Debenzylation, using a mixture of potassium *tert*-butoxide and an O_2-saturated solution in DMSO/THF,[30,31] gave indazole **5**, which was resolved by chiral preparative supercritical fluid chromatography[32] into the presumptive 1*R*,2*S* (**5a**) and 1*S*,2*R* (**5b**) enantiomers. Racemic **5** was smoothly iodinated to give **17**; this material was identical in all respects to a sample of racemic **17** generated by treatment of **1** with sulfoxonium ylide, as previously described.[24] The racemic (1*R*,2*S*)-2-(3-iodo-1*H*-indazol-6-yl)-5′-methoxyspiro-[cyclopropane-1,3′-indolin]-2′-one **18** was similarly available via the cyclopropanation of alkene **1b**.

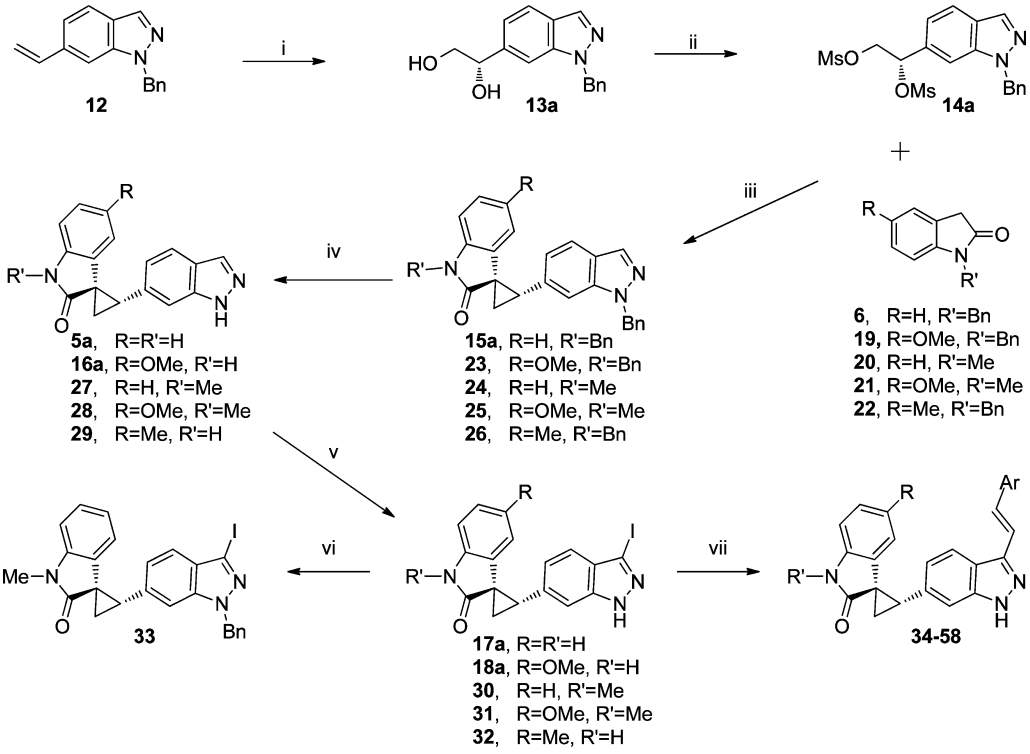
The racemic iodoindazole **17** was resolved as above into the presumptive 1*R*,2*S* (**17a**) and 1*S*,2*R* (**17b**) enantiomers (Scheme 3); enantiomers **18a** and **18b** were similarly obtained. The tentative assignment was based on correlation of optimal activity (Table 1) with the co-complex X-ray structure given in our previous paper.[24] Subsequent conversion of **17a** and **17b** to compounds **3a** and **3b** and compounds **18a** and **18b** to compounds **4a** and **4b**, respectively, was effected by Suzuki–Miyaura reaction.

With these results in hand, preparation of chiral diol **13a** (*S*-enantiomer), predicted to yield the desired isomer **5a**, was initiated (Scheme 4). Sharpless asymmetric dihydroxylation of **12** using AD-mix-α generated diol **13a** as the *S*-enantiomer in high enantiomeric excess (>98%).[33] The diol was subsequently converted to the chiral dimesylate **14a** in high yield. The key reaction, i.e., double S_N2 displacement of the dimesylate **14a** with the anionic oxindole **6**, proceeded cleanly to give the protected spirocyclopropane **15a** with high enantioselectivity (≥98% ee by chiral HPLC) as the presumptive 1*R*,2*S* enantiomer. Similarly, compounds **23**–**26** were prepared by treatment of **14a** with oxindoles **19**–**22**, respectively.

Attempted removal of the benzyl protecting groups of compound **15a**, under catalytic hydrogenation conditions, resulted

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX–XXX

Scheme 4. Stereoselective Synthesis of (1*R*,2*S*)-2-(1*H*-Indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-ones[a]



[a]Reagents and conditions: (i) $(DHQ)_2PHAL$, $K_2OsO_4{\cdot}2H_2O$ (0.5 mol %), $K_3Fe(CN)_6$, K_2CO_3, *t*-BuOH/H_2O, 0 °C, 5 h; (ii) MsCl, TEA, CH_2Cl_2, 0 °C; (iii) NaH (60% in oil), THF; (iv) DMSO, *t*-BuOK, THF, O_2; (v) I_2, K_2CO_3, DMF; (vi) BnBr, DCM, 50% aq KOH, TBAB; (vii) ArCH=CHBpin, 2:1 PhMe/EtOH, 1 M Na_2CO_3, $Pd(PPh_3)_4$ (5 mol %), microwave 120 °C, 2 h or 1 M Na_2CO_3, LiCl, $Pd(PPh_3)_4$ (2.5 mol %), dioxane, reflux, 18 h.

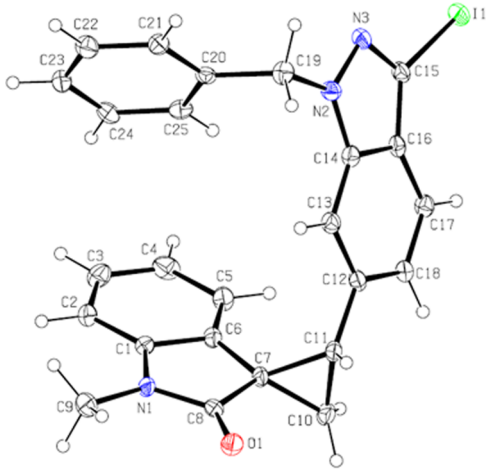


Figure 1. ORTEP diagram for compound **33** X-ray structure showing the 1*R*,2*S* stereochemistry.

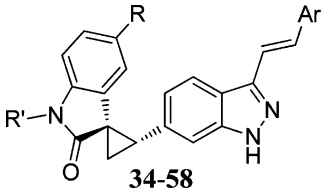
in partial ring-opening of the cyclopropane. However, the benzyl groups were removed cleanly from compounds **15a** and **23**–**26** using the conditions developed for the racemate **15**, i.e., *t*-BuOK in an O_2 saturated solution of THF/DMSO. Compound **5a**, so prepared, was identical to the material obtained by preparative chiral chromatography (Scheme 3). Subsequent iodination of intermediates **5a**, **16a**, and **27**–**29** gave **17a**, **18a**, and **30**–**32**, respectively, in yields ranging from 45% to 95%. Enantiomeric excesses were determined at this stage of the syntheses (chiral HPLC) and were found to be uniformly ≥98%. Compounds **17a** and **18a** were identical in all respects to the materials prepared in Scheme 3 via chiral prep-HPLC. At this stage, compound **30** was benzylated and the product of the reaction **33** was recrystallized; single crystal X-ray structure determination was performed on this material (vide infra). PLK4 inhibitors **34**–**58** were prepared via Suzuki–Miyaura coupling of iodoindazoles **17a**, **18a**, and **30**–**32** with the vinyl boronic acid pinacol esters in moderate yields.

Cyclopropane Stereochemistry. Our initial assignments of stereochemistry at positions 1 and 2 of the cyclopropane ring were based on molecular modeling predictions, wherein the 1*R*,2*S* enantiomer scored up to 2 orders of magnitude higher than the 1*S*,2*R*. This tentative assignment was corroborated by the PLK4 co-complex X-ray structure given in the previous paper and the chemistry outlined in Scheme 4 which is predicted to yield the 1*R*,2*S* isomer preferentially. This assignment was confirmed by an X-ray crystallographic study of **33** (see Supporting Information), which indicates that the 1*R*,2*S* stereochemistry of the cyclopropyl enantiomer is derived from the vicinal (*S*)-diol **13a** via the double S_N2 displacement reaction of **14a** (Scheme 4). The corresponding ORTEP diagram is shown in Figure 1.

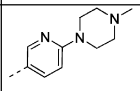
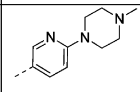
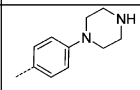
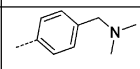
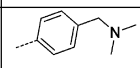
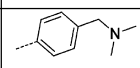
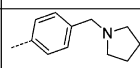
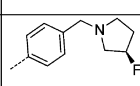
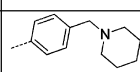
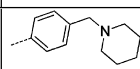
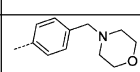
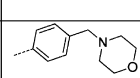
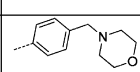
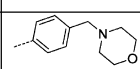
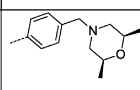
The 2*S* stereochemistry is set by the S_N2 inversion of the benzylic mesylate as anticipated (Scheme 2). The induction at the spiro position of the cyclopropane to give 1*R*, virtually exclusively, was less certain. It would appear that hydrophobic collapse and/or a π-stacking interaction[34] serves to align the indolinone and indazole in such a way to ensure that ring closure yields the desired isomer, i.e., the left fork of the proposed mechanism of Scheme 2. Bis-electrophiles analogous to **14a** react preferentially at the benzylic position.[35−37] However, an alternative reaction course, in which the first

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

Table 2. In vitro Activity of (1*R*,2*S*)-2-(1*H*-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-ones PLK4 Inhibitors

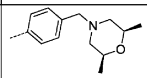
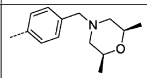
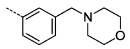
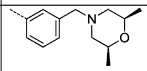
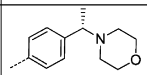
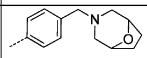
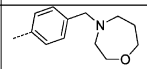
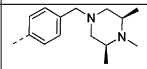
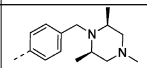
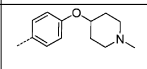
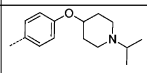


34-58

Entry	R	R′	Ar	PLK4 IC_{50} (nM)	Breast Cancer GI_{50} (μM)				HMEC GI_{50} (μM)
					MDA-MB-468	MCF-7	HCC-1954	MDA-MB-231	
3	NA			1.1	<0.01	<0.01	1.9	12	28
4	NA			2.2	0.006	0.01	7.3	14	ND
34	H	H		3.4	0.06	2.0	7.5	1.0	4.4
35	OMe	H		0.75	<0.001	<0.001	0.002	2.6	47
36	H	Me		1.3	0.001	0.02	0.005	0.6	ND
37	H	H		0.9	0.002	0.020	0.26	0.023	21
3a	H	H		0.59	<0.001	0.25	ND	0.82	19
38	OMe	H		0.37	<0.001	0.0001	5	2.8	41
39	H	Me		1.4	0.002	0.0015	0.007	0.75	8.0
40	H	H		0.9	<0.001	0.37	0.026	0.25	3.6
41	H	H		3.8	0.0067	34	0.022	5.6	7.9
42	H	H		0.9	<0.001	<0.001	5	2.2	20
43	OMe	H		0.6	<0.001	<0.001	5	1.6	50
44	H	H		1.4	0.0042	0.80	<0.001	5	50
45	Me	H		3.1	0.013	<0.01	<0.01	22	9.3
4a	OMe	H		0.9	<0.0001	0.0001	0.25	<0.001	>50
46	OMe	Me		1.3 ± 0.6	<0.01	<0.01	<0.01	20	>30
47	H	H		7.4 ± 2.1	0.01	0.017	0.008	6.5	8.4

dx.doi.org/10.1021/jm5005336 | *J. Med. Chem.* XXXX, XXX, XXX−XXX

Table 2. continued

Cmpd.	R	R'	Ar	PLK4 IC_{50} (nM)	Breast Cancer GI_{50} (μM)				HMEC GI_{50} (μM)
					MDA-MB-468	MCF-7	HCC-1954	MDA-MB-231	
48	OMe	H		2.8 ± 1.4	0.006	0.008	0.005	8.6	9.0
49	OMe	Me		5.7	0.029	0.021	0.015	50	>30
50	H	H		7.6	0.13	0.35	1.6	2.2	50
51	H	H		20	1.1	1.3	0.084	9.6	ND
52	H	H		1.5	0.01	0.05	0.01	1.0	9.6
53	H	H		5.2	0.061	0.17	0.053	4.1	ND
54	H	H		1.7	0.002	1.0	1.8	3.0	ND
55	H	H		0.64	0.01	2.5	1.3	0.5	ND
56	OMe	H		0.39	0.01	2.5	2.3	2.2	ND
57	H	H		1.1	0.002	2.4	0.77	0.92	ND
58	H	H		1.5	0.005	0.067	0.007	1.6	4.1

displacement occurs on the primary position followed by inversion at the benzylic position, cannot be ruled out. The stereochemical consequences of this sequence would be the same as long as the latter reaction is controlled by an S_N2 process, and the aforementioned π-stacking is operative.

Table 1 summarizes the PLK4 activity for three racemates, **17**, **3**, and **4**, and their constituent enantiomers. It is clear that the 1*R*,2*S* stereoisomer 17**a** is substantially more active against PLK4 than the 1*S*,2*R* stereoisomer **17b** (~100 fold) and that the active isomer is 2 times more active than the racemate. This preference is also seen in the more elaborated and potent inhibitors **3** and **4** and their constituent enantiomers, **3a**,**b** and **4a**,**b**, respectively. The cell growth inhibitory activity also reflects this SAR; the PLK4 active isomers are 1−2 orders of magnitude more effective at attenuating MDA-MB-468 breast cancer cell growth. Subsequent lead optimization efforts were focused on the 1*R*,2*S* isomer.

Lead Optimization. Much of the SAR that had been developed on the (*E*)-3-((1*H*-indazol-6-yl)methylene)indolin-2-ones[17] was transferable to the racemic 2-(1*H*-indazol-6-yl)-spiro[cyclo-propane-1,3′-indolin]-2′-ones[24] and, as anticipated, to the optically active version as well. This is made clear by the entries of Table 2, which indicate that excellent biochemical potency was maintained by the chiral PLK4 inhibitors. Trends previously established were recapitulated in that substitution of the indolinone with a methoxy group at the 5′ position was generally beneficial, as supported by the matched pair **47** and **48**. The objective of the work going forward was to optimize the drug-like characteristics of the compounds in hopes of identifying an orally bioavailable development candidate for the treatment of solid tumors. Our efforts were centered on maintaining the in vitro potency of the compounds while maximizing PK properties by fine-tuning the indolinone substituents R and R′ and the aromatic moiety Ar.

Cell growth was determined by measuring total protein content by SRB assay after 5 days of treatment. In accordance with other mitotic kinase inhibitors,[38,39] PLK4 inhibitors require treatment times of ≥4 days for their effects to manifest.[40,41] Figure 2 illustrates the effect of a representative PLK4 inhibitor, namely compound **47**, on the growth inhibition of four breast cancer cell lines (HCC1954, MCF-7, MDA-MB-231, and MDA-MB-468). The plot shows % survival (normalized to control) versus log

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

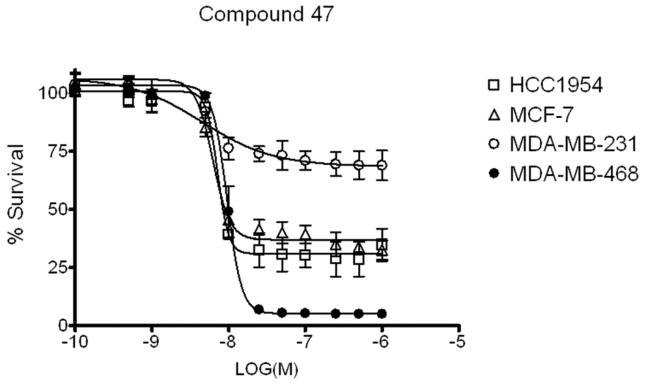


Figure 2. SRB dose–response curves for HCC1954, MCF-7, MDA-MB-231, and MDA-MB-468 cells upon treatment with compound **47** for 5 days.

concentration of compound **47** in molar (M) and illustrates the differential cellular responses to compound treatment. In related work, we have evaluated the effect of our PLK4 inhibitors on growth attenuation in panels of cancer cell lines, including breast. Distinct outcomes have been observed: polyploidization followed by cell death (e.g., MDA-MB-468), polyploidization followed by cell arrest (e.g., MDA-MB-231), and an intermediate effect.[40,41] The sensitivity of the most responsive cell lines (MDA-MB468 followed by HCC1954 and MCF-7) to compound **47** is very similar, i.e., the inflection points for the dose response curves are around 10 nM in each case. It is difficult to accurately determine the sensitivity of the MDA-MB-231 cell line in that the survival is high (~70%) and the inflection is gradual and indistinct. The GI_{50}s, i.e., concentrations of PLK4 inhibitors required to inhibit growth of these breast cancer cell lines by 50% of the maximal response has been captured in Table 2.

Our first publication on PLK4 inhibitors[17] had demonstrated the attributes of the 6-(4-methylpiperazin-1-yl)pyridin-3-yl aryl group vis a vis the pyridinyl aryl group (**34**), and this was recapitulated in the chiral 2-(1*H*-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-ones. The basic piperazine group in this solvent accessible region of the molecule is associated with enhanced cell activity with the side benefit of increased aqueous solubility. Compounds **35** and **36** are potent against PLK4 and, excepting MDA-MB-231, are effective in attenuating breast cancer cell growth across the panel. The latter compound, by methylating the indolinone nitrogen, has the benefit of removing an H-bond donor and potentially increasing permeability. Inspection of our binding models had suggested that this substitution would be tolerated. Although *N*-methylation removes a potential H-bond with Gln-160, this interaction is solvated and has little thermodynamic value. Because of the *N*-Me/carbonyl repulsion, the molecule is pushed away from Gln-160; deeper insertion of the 5′-methoxy group into the hydrophobic pocket results, which appears to compensate somewhat for the loss of a weak H-bond. PLK4 inhibitor **37** was prepared in an effort to remove the potential liability of the pyridyl nitrogen; the latter maintains excellent cell activity and solubility. Compounds **34**, **35**, and **37** were taken forward into mouse PK studies.

An alternate motif, described in the preceding work,[24] is the 4-((dialkylamino)methyl)-phenyl group, found in compound **3a**. This moiety also imparts improved aqueous solubility, especially at acidic pH (pK_a = 8.8)[42] and is generally associated with potent inhibition of PLK4. A series of analogues were prepared in which this basic motif was retained, i.e., compounds **38**–**43**; biochemical potency ranged an order of magnitude with the best inhibitors at the subnanomolar level. The 5′-methoxy compound **38** was the most potent; in contrast, appending a methyl group at the N-1 position of the indolinone decreased potency by 2-fold, compound **39**. The potential benefits of *N*-methylation (described above for **36**) were realized in that compound **39** was the most effective cell growth inhibitor of the triplet of compounds incorporating the 4-((dialkylamino)methyl)-phenyl group.

Replacement of the dimethyl amine with cyclic variants gave compounds **40**–**43**; potency against PLK4 was generally maintained as was attenuation of the growth of MDA-MB-468 cells. Curiously, the pyrrolidines (**40**, **41**) were effective against HCC-1954 cells while the piperidines (**42**, **43**) inhibited the growth of MCF-7 cell line more strongly; we have no explanation for this empirical observation. Representatives, **41** and **43**, were tested in mouse PK studies.

The promise of the cyclic inhibitors was explored in additional variations on this theme, namely the morpholine analogues **4a** and **44**–**53**, the oxazepane **54**, and the piperazines **55** and **56**. The inclusion of the oxygen in this ring system reduces the pK_a of the benzylic nitrogen from 8.9 to 7.1;[42] we reasoned that modulation of the basicity could enhance permeability and result in improved cell activity and PK parameters. These inhibitors potently inhibited PLK4 and, excepting MDA-MB-231, effectively inhibited the growth of the breast cancer cells. We noted that the meso dimethyl analogues **47**–**49** were somewhat less effective against PLK4, but good activity against the cell panel was maintained. However, the 1,3-phenyl substituted analogues **50** and **51** were less active than their 1,4 substituted counterparts and were eliminated from further consideration. The methyl, bicylic, and ring expanded analogues **52**, **53**, and **54**, respectively offered no advantage over the parent compound **44**.

Inclusion of nitrogen into the ring system, i.e., piperazines **55** and **56**, shifts the most basic center to the distal nitrogen and yields compounds with relatively high pK_as (8.4–8.8).[42] These compounds inhibited PLK4 potently but weakly attenuated growth of the HCC-1954 and MCF-7 cell lines. In an effort to maintain the topology of the morpholinomethyl moiety while moving the basic center to the distal ring position, (alkylpiperidin-4-yl)oxy compounds **57** and **58** were prepared. The latter compound in particular had potent in vitro activities and was tested more extensively in eADME and in vivo assays. It should be noted that the growth inhibitory effect of compounds on human mammary epithelial cells (HMEC GI_{50}) was measured (Table 2). The ability of the PLK4 inhibitors to inhibit the growth of these cells was poor and generally about 3 orders of magnitude less potent than the effects seen on breast cancer cell lines such as MDA-MB-468.

eADME and Mouse Plasma Levels. Cytochrome P450, microsomal stability ($T_{1/2}$), and mouse plasma levels (PO dosing) were obtained for selected inhibitors, as given in Table 3. Compounds were routinely tested against the 1A2 and 2D6 isoforms, however, no inhibition was observed (data not shown). As seen for the alkene-linked compounds,[17] the CYP450 IC_{50} values were generally lower for 2C9 and 2C19 as opposed to other isoforms. Overall, the IC_{50}s against CYP450s were micromolar or higher, including the all-important 3A4 isoform. In some instances, values dipped significantly below the single-digit micromolar range (entries **49**, **52**, and **56**) and these compounds were not

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX–XXX

Table 3. eADME and Mouse Plasma Levels for Selected PLK4 Inhibitors[a]

	CYP_{450} IC_{50} (μM)				microsomal $T_{1/2}$ (min)				mouse plasma levels (PO @ 25 mg/kg)	
entry	2C9	2C19	3A4 DBF	3A4 BFC	m	r	d	h	C_{max} (μg/mL)	AUC (μg·h/mL)
35	2.0	1.5	5.6	~10	6	ND	ND	11	0.2	0.38
36	3.8	1.2	2.9	0.3	7	ND	ND	27	0.7	1.6
37	1.7	0.9	2.8	ND	19	ND	ND	46	0.120	0.34
3a	>1	>1	~10	~5	13	ND	ND	>60	0.11	0.26
38	3.5	2.0	7.5	ND	22	ND	ND	36	0.3	0.64
39	2.6	1.9	>1	2.3	12	ND	ND	44	0.51	1.1
41	0.9	1.5	~10	>1	17	ND	ND	52	0.79	1.8
43	3.4	1.4	5.4	>1	40	ND	ND	49	0.51	1.3
44	0.7	0.8	2.7	6.3	15	7.3	4.7	45	3.5	10
45	1.0	1.2	6.2	3.0	13	9	9.5	56	2.1	5.5
4a	0.8	1.2	6.4	2.4	12	8.3	9.4	41	3.5	9.5
46	1.0	1.0	6.0	20	24	6.4	13	37	5.9	17
47	0.8	1.7	5.0	5.1	32	>60	>60	>60	3.7	10
48	0.94	1.4	4.6	3.1	24	53	43	>60	2.2	9.8
49	0.44	0.76	>1.0	~10	10	18	>60	>60	3.4	6.8
52	0.44	0.9	11	0.45	7.2	10	6.8	53	3.3	16
56	0.56	0.81	1.6	~10	38	49	39	>60	1.3	2.2
58	2.4	1.9	1.6	>1	30	>60	>60	>60	0.51	1.4

[a]ND: not determined.

taken forward. Mouse microsomal $T_{1/2}$ was often less than 30 min for the earlier entries, but the half-lives were longer for the later entries; microsomal $T_{1/2}$ was also longer in the presence of human microsomes as compared to mouse. As a predictor of in vivo performance in the putative toxicology species (rat and dog), we measured mouse plasma levels obtained upon oral dosing. Mouse was used for these preliminary exposure studies being readily available, compound sparing, and the species of choice for the subsequent xenografts.

The first eight entries in Table 3 attained modest plasma levels in mouse, with the highest exposure being achieved by the *N*-Me indolinone analogues **36** and **39** and the cyclic inhibitors **41** and **43**. It is possible that the increased lipophilicity of these entries enhances permeability, however, these effects are modest. In contrast, the large increase in exposure for the morpholines **4a** and **44**−**49** is unmistakable. We postulate a role for the ring oxygen in attenuating the basicity of the nitrogen (pK_a = 7.1), thus striking a balance between enhanced solubility at low pH and good permeability at physiological pH. As a group the morpholino-methyl compounds achieved exposures an order of magnitude higher than their dialkylamino-methyl comparators. The more basic piperazinyl and (alkylpiperidin-4-yl)oxy moieties, as represented by compounds **56** and **58**, respectively, showed similarly lower exposures.

During the course of the mouse plasma studies, we modeled the ability of early ADME measures to predict the performance of our compounds in vivo. Figure 3 gives the correlation of logAUC (experimental) with logAUC (predicted) for PO dosing of PLK4 inhibitors in mice. QSAR analysis established that two independent variables, namely mouse microsomal $T_{1/2}$ and log$D_{7.4}$, can describe predicted logAUC with an R^2 of 0.43 and RMSE of 0.43. The correlation is low but not unexpected when modeling in vivo data. The level of variance observed indicates that additional factors could be important in describing exposure of our PLK4 inhibitors in mice, such as the involvement of phase II metabolism, which would not be captured by microsomal measurements. It appears that the microsomal metabolism was of some value in guiding compound optimization but of limited applicability in predicting overall in vivo performance.

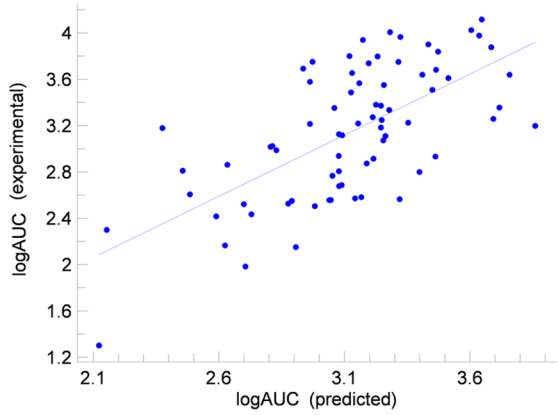


Figure 3. Correlation of logAUC (experimental) with logAUC (predicted) for PO dosing of PLK4 inhibitors in mice. QSAR analysis established that two independent variables (mouse microsomal $T_{1/2}$ and log$D_{7.4}$) describe logAUC (predicted), with an R^2 of 0.43 and RMSE of 0.43 (n = 74).

Morpholines with a desirable combination of in vitro and in vivo properties, namely compounds **4a** and **44**−**48**, were advanced to rat and dog pharmacokinetic studies (Table 4). Compounds **4a**, **44**, and **45** had relatively high clearance rates in rat; **4a** had a short dog $t_{1/2}$ and **44** had a low oral bioavailability in dog. Compound **45** had the additional burden of being relatively difficult to make, a feature shared by all compounds with a methyl group at the 5′ position of the indolinone. Thus, a short list of three emerged from these studies as compounds of high interest: **46**, **47**, and **48**. These inhibitors were tested in an expanded cancer cell line panel including lung, colon, and ovarian cancers (Table 5). All three compounds had very similar profiles, with responses more dependent on the cancer cell line tested as opposed to the compound per se; nonetheless, the overall rank order appeared to be in line with the inherent potency of the compounds against PLK4.

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

Table 4. Rat and Dog Pharmacokinetics for Selected PLK4 Inhibitors

		4a	44	45	46	47	48
rat IV (1 mg/kg)	Cl (L·h/kg)	4.0	3.2	3.5	1.9	0.84	1.9
	$t_{1/2}$ (h)	1.1	1.4	1.6	1.3	3.9	2.0
	AUC_{INF} (ng·h/mL)	250	312	285	553	1234	534
rat PO (5 mg/kg)	AUC_{INF} (ng·h/mL)	272	478	410	688	2807	767
	oral BA (%)	22	31	28	25	46	21
dog IV (1 mg/kg)	Cl (L·h/kg)	2.5	2.0	1.4	1.6	0.50	0.96
	$t_{1/2}$ (h)	0.87	3.2	7.0	3.0	4.5	2.1
	AUC_{INF} (ng·h/mL)	402	515	795	648	2050	1054
dog PO (5 mg/kg)	AUC_{INF} (ng·h/mL)	1423	250	1139	1030	4987	3460
	oral BA (%)	71	9.7	29	32	49	66

Table 5. PLK4 K_i and Growth Inhibition Data for 46, 47, and 48 in an Expanded Panel of Cancer Cell Lines

		cancer cell line GI_{50} (μM)							
		breast			lung	ovarian	colon		
entry	PLK4 K_i (nM)	SKBr-3	Cal-51	BT-20	A549	OVCAR-3	SW620	Colo-205	HCT116+/+
46	0.10 ± 0.04	6.5	0.11	0.055	0.004	0.007	0.60	0.007	0.005
47	0.73 ± 0.28	7.2	0.091	0.09	0.009	0.015	0.35	0.057	0.008
48	0.26 ± 0.10	5.3	0.26	0.058	0.005	0.018	0.38	0.017	0.004

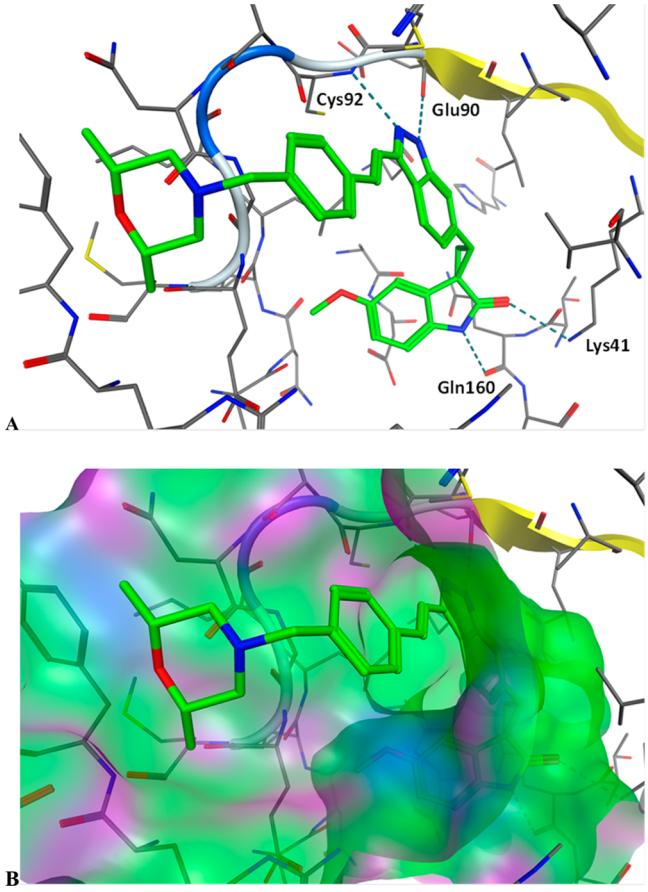


Figure 4. Binding mode for compound **48** generated by GlideXP docking in the PLK4 (PDB code 4JXF) active site. (A) Top panel shows H-bonds from indolinone carbonyl and NH to Lys41 and Gln160 respectively and H-bonds from indazole to Glu90 and Cys92 of the hinge. (B) Bottom panel shows a similar view with a surface representation on the protein; morpholine is at protein−water interface; the OMe group nestles in a groove bounded by the protein backbone and side chains.

PLK4 Binding Model. A model of compound **48** in the PLK4 active site is shown in Figure 4 and is representative for inhibitors of this class. Key interactions, shown in Figure 4A, include two H-bonds from the indazole to the hinge (Glu90 and Cys92) and two H-bonds from the indolinone carbonyl and NH to Lys41 and Gln160, respectively. Figure 4B shows a similar view with a surface representation on the protein; the morpholine is at the protein−water interface. There is extensive hydrophobic contact between the enzyme and the ligand, especially at the aromatic rings. The methoxy group nestles in a groove bounded by the protein backbone and the side chains of Leu143 and Glu96.

Kinase Selectivity. The short list of PLK4 inhibitors was profiled against other members of the Polo-like kinase family; none of the compounds significantly inhibited PLK1, 2, or 3 at a concentration of 50 μM. In an effort to distinguish between compounds, the short list was tested (Millipore radiometric assay) against a large panel of kinases (see Supporting Information). Only a subset of the kinases (48 of 274) was inhibited above 50% at 0.1 μM by at least one compound. This data is represented as a heat map in Figure 5; the PLK4 inhibitor **48** was clearly more selective than compound **46**, followed by **47**. For compound **48**, biochemical (IC_{50}) and cellular (EC_{50})[40,41] inhibition constants were determined for those human kinases which were significantly inhibited (Table 6). There is an order of magnitude separation between the EC_{50}s for PLK4 (12 nM) and the off-target kinases namely AURKA, AURKB/INCENP, TIE2/TEK, TRKA, and TRKB. A kinetic analysis of compound **48** (Table 5 and Supporting Information) confirmed the potent (K_i = 0.26 ± 0.10 nM) and ATP competitive nature of the binding, not surprising given the X-ray structures of related compounds.

Although inhibitory effects in cellular contexts were demonstrated for TrkA, TrkB, and Tie2/TEK, these kinases do not have expression patterns or biological activity that would account for the effects of the PLK4 inhibitor **48** on the cells tested in vitro. TrkA and TrkB are highly expressed in neuroblastomas,[43] whereas Tie2/TEK is overexpressed in the microvasculature of solid tumors.[44] AURKB is present in all proliferating cells and an integral component of the chromosome passenger protein complex (CPPC), thus its inhibition could explain some of the observed effects of **48** such as the accumulation of polyploid

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

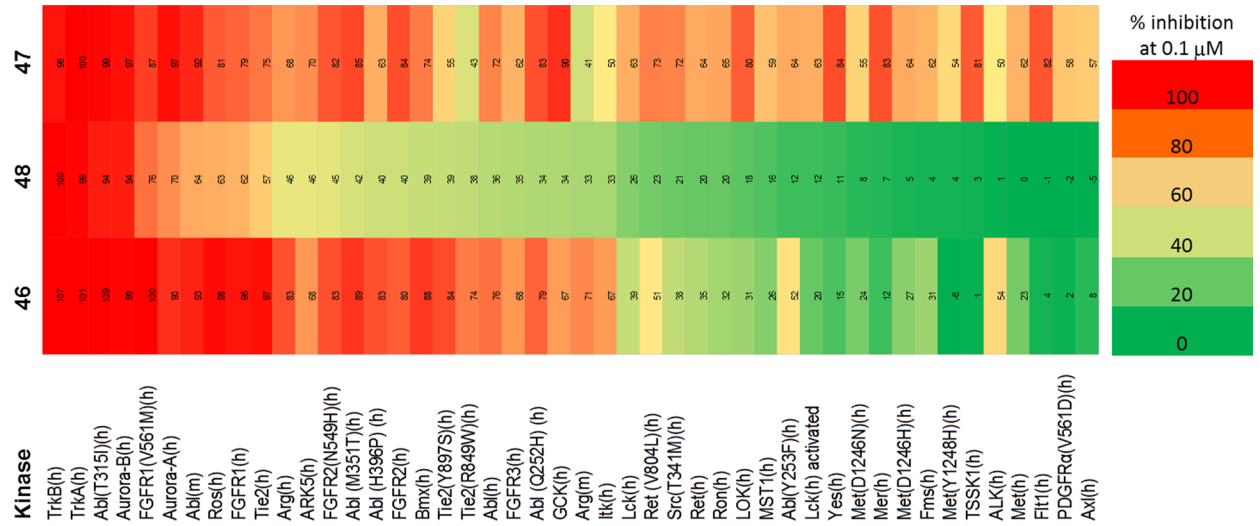


Figure 5. Heat map showing % inhibition of kinases at a screening concentration of 0.1 μM by PLK4 inhibitors **46**, **47**, and **48**. Only kinases inhibited >50% by at least one compound (46 of 274) are represented.

Table 6. Biochemical (IC_{50}) and Cellular (EC_{50}) Binding Constants for Compound 48

kinase	IC_{50} (nM)	EC_{50} (nM)	kinase	IC_{50} (nM)	EC_{50} (nM)
PLK4	2.8	12	TIE2/TEK	22	117
AURKA	140	510	TRKA	6	84
AURKB/ INCENP	98	102	TRKB	9	88

cells.[40,41] Cellular effects of PLK4 inhibition are observed (e.g., inhibition of centriole duplication),[40,41] and compound **48** is 8.5-fold selective for PLK4 over AURKB in cells. However, we cannot rule out that inhibition of AURKB, or other off-target kinases, contribute to the activity of compound **48**.

In Vivo Efficacy. Mouse xenograft studies were performed with the short list, chosen from three pathologies, namely, breast (MDA-MB-468), colon (SW620), and lung (A549); these clean cell lines were well established in our laboratories, demonstrating reproducible tumor growth rates in mice. The compounds were given orally at a high (9.4 mg/kg) and low (3.0 mg/kg) dose, once a day, for 21 days. Figure 6 shows the results for a representative study with compounds **47** and **48** in the breast MDA-MB-468 xenograft model. All doses were tolerated in that none of the animals were lost during the course of the experiment (21 days) and none of the mice lost more than 20% of their body weight. A dose response was seen for both compounds, and all four treatment arms reached statistically significant response vis a vis control ($p < 0.05$). Table 7 summarizes the results for the shortlisted compounds in the three xenograft models. Note that all three compounds were efficacious in the models tested, albeit to varying degrees; there was a robust response in the SW620 xenograft which would not have been predicted by the in vitro results (Table 5). The lone exception was compound **46** in the A549 model; the high dose was not tolerated and all animals in this arm were lost. In summary, we deemed these results supported our interest in the shortlisted compounds as potential anticancer agents but were not discriminatory.

There are theoretical reasons to suppose that antimitotic agents can better exert their effects on tumor growth inhibition by employing an intermittent dosing regimen,[45] and some chemotherapeutic agents perform optimally on such schedules.[46−48] Accordingly, the maximum tolerated dose (MTD) for compounds **46**, **47**, and **48** for a 1-on/3-off schedule were estimated to be 39, 36, and 39 mg/kg, respectively. Thus, we were able to increase the total compound dosed over the 21 day experiment from 197.4 mg/kg for 9.4 mg/kg (QD regimen) to 252 and 273 mg/kg for the 36 and 39 mg/kg (1-on/3-off regimen) respectively. The short list was dosed at MTD and 2/3MTD in an HCT116 xenograft model (Figures 7A,B), with the last dose on day 21. PLK4 inhibitor **48** was the most effective followed by compounds **46** and **47**; the maximal response was seen at day 25 (98, 90, and 78% TGI, respectively, at the MTD). All dosing arms were tolerated. The experiment continued to day 35 (93% TGI for compound **48** at 39 mg/kg); at this point, the control tumors had grown to a volume such that the animals needed to be taken off study and sacrificed.

Compound 48. Compound **48** represents an optimized structure from the (1*R*,2*S*)-2-(3-((*E*)-4-((dialkylamino)methyl)-styryl)-1*H*-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one class of PLK4 inhibitors. Although having comparable in vitro potency to many other inhibitors listed in Table 2, the morpholino group confers a high level of oral exposure not shared by the compounds incorporating more basic dialkylamino moieties. The two methyl substituents on the morpholine confer additional benefit in that clearance in rat and dog is significantly reduced. The methoxy substituent on the indolinone ring is associated with increased potency and selectivity as evidence by the head to head comparison of compounds **47** and **48**. The advantages of compound **48** culminate in its superior performance in mouse xenografts. On the basis of the cumulative results (vida supra) and additional preclinical data,[40,41] compound **48** was selected for IND enabling studies.

■ CONCLUSIONS

We have verified the configuration of the most active stereoisomer of the (2-(1*H*-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-ones to be 1*R*,2*S* and have described a novel synthetic method to obtain the same, with high enantiomeric and diastereomeric purity. This breakthrough set the stage for systematic optimization of this series to identify the morpholinomethyl-styryl derivatives with nanomolar activity against PLK4 and concomitant inhibitory effects against a panel of breast cancer cell

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

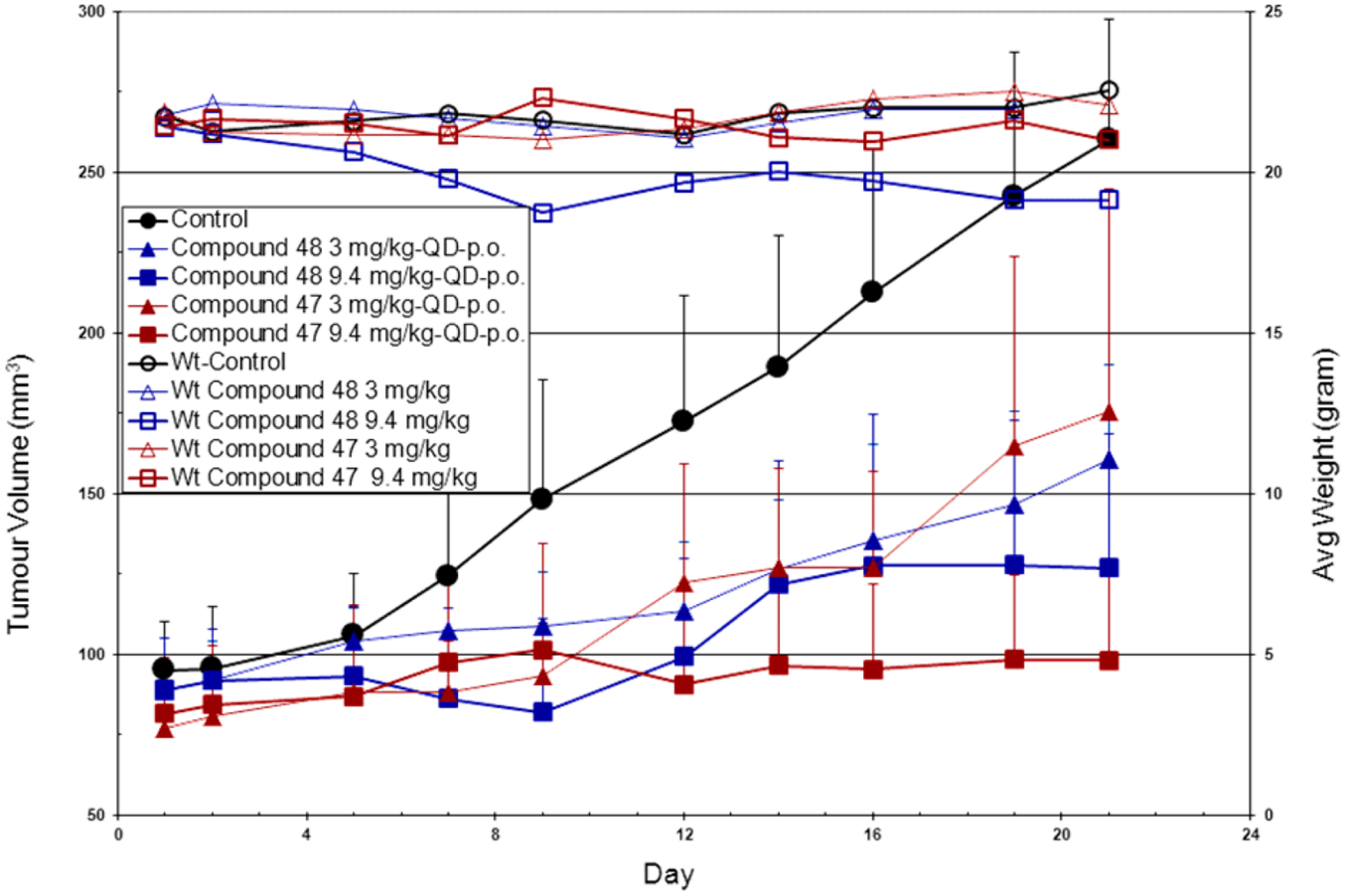


Figure 6. Tumor volume and average weight vs days of treatment with compounds **47** and **48** in an MDA-MB-468 mouse xenograft model (PO QD dosing).

Table 7. Mouse Xenograft Results for PLK4 Inhibitors 46, 47, and 48

	percent tumor growth inhibition					
	MDA-MB-468		SW620		A549	
entry	dose 3 mg/kg	dose 9.4 mg/kg	dose 3 mg/kg	dose 9.4 mg/kg	dose 3 mg/kg	dose 9.4 mg/kg
46	61[a]	96[a]	47[a]	77[a]	46[a]	ND[b]
47	45	90[a]	57[a]	80[a]	44[a]	79[a]
48	57[a]	77[a]	7	86[a]	32	70[a]

[a] $p < 0.05$. [b] All animals in this arm were lost during the study.

lines. These compounds demonstrated high exposure upon oral dosing in mice, and a subset was taken forward into rat and dog PK studies from which a short list of three compounds was selected for detailed study.

Compounds **46**, **47**, and **48** are single-digit nanomolar PLK4 inhibitors and selective against other members of the PLK family (>10 μM). The shortlisted compounds attenuate the growth of a variety of breast, colon, and ovarian cancer cell lines and are efficacious in mouse xenograft models of tumor growth. Compound **48** is the most selective shortlisted compound as determined by a radiometric assay against a panel of 274 human kinases. Upon intermittent oral dosing, in a mouse model of colon cancer, compound **48** was an effective inhibitor of HCT116 tumor growth and was well tolerated. On the strength of the data presented herein, and additional preclinical studies,[40,41] compound **48** (designated CFI-400945) was selected for clinical development. IND enabling studies have been completed and this compound has entered phase I human trials in Canada and the United States.

EXPERIMENTAL SECTION

Computational Methods. The computational processes, model refinement, and QSAR-type analyses were performed using the MOE software.[49] The PLK4 co-complex X-ray structure, described in the previous paper,[24] was used for the docking experiments described herein. Ligand preparation, conformer generation, and docking experiments were as previously described.[17]

Synthetic Chemistry. *General Experimental Methods.* Commercially available starting materials, reagents, and solvents were used as received. In general, anhydrous reactions were performed under an inert atmosphere such as nitrogen or argon. Microwave reactions were performed using a Biotage Initiator microwave reactor. Reaction progress was generally monitored by TLC using Merck silica gel plates with visualization by UV at 254 nm by analytical HPLC or by LCMS (Bruker Esquire 4000). Flash column chromatographic purification of intermediates or final products was performed on a Biotage Isolera One purification system using SNAP KP-sil cartridges. Crude reactions were partially purified using Waters PoraPak Rxn CX cartridges. Preparative reverse-phase HPLC purification was performed on a Varian PrepStar model SD-1 HPLC system with a Varian Monochrom 10 μ C-18 reverse-phase column. Elution was performed using a gradient of 10% MeOH/water to 90% MeOH/water (0.05% TFA) over a 40 min period at a flow rate of 40 mL/min. Fractions containing the desired material were concentrated and lyophilized to obtain the final products. All reported yields were not optimized. Proton NMRs were recorded on a Bruker 400 MHz spectrometer, and mass spectra were obtained using a Bruker Esquire 4000 spectrometer. Chiral SFC separations were performed by Lotus Separations, Princeton, NJ.

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

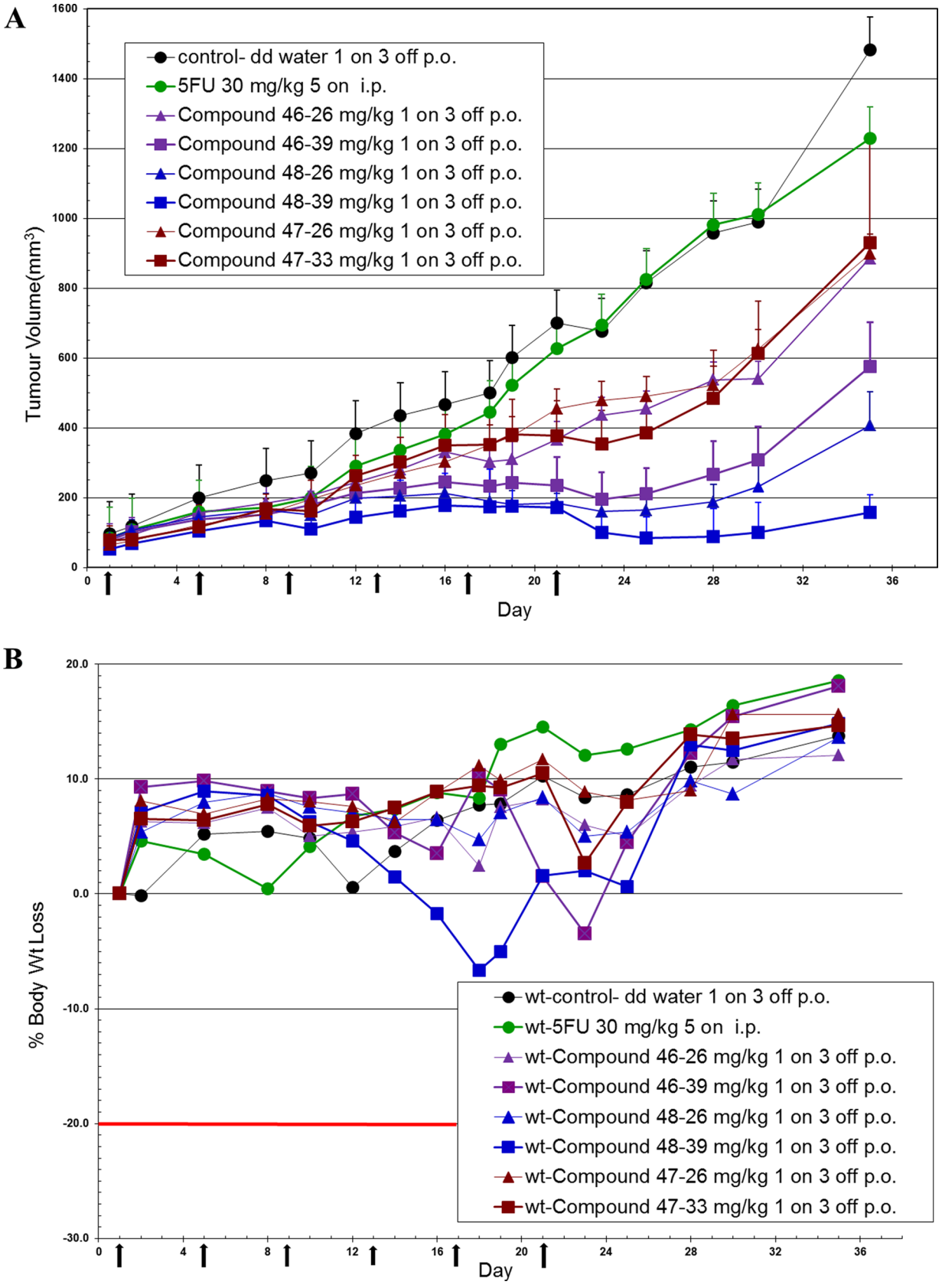


Figure 7. HCT-116 mouse xenograft model. Solid arrows indicate treatment days with compounds **46**, **47**, and **48** (1 on 3 off, PO); 5-fluorouracil was given at 50 mg/kg IP for 5 days. (A) Top panel shows tumor volume vs day following treatment initiation. (B) Bottom panel shows percent mean body weight loss vs day following treatment initiation. The solid arrows indicate dosing events.

Optical rotation was measured on an Optical Activity AA-55 polarimeter. Compound purity by analytical HPLC (UV detection at λ = 270 nm) was performed on a Varian Prostar HPLC or Shimadzu Prominance HPLC, using a 100 mm × 4.6 mm Phenomenex Luna 3 μ

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

C-18 column. Elution was performed using a gradient 10% MeOH/water to 90% MeOH/water (0.05% TFA) between 1 and 10 min at a flow rate of 1 mL/min. Purity is reported as area percent; the purity of all final compounds was shown by HPLC to be ≥95%.

Low resolution mass spectra (MS ESI) were obtained using a Bruker Esquire 4000 spectrometer under electron spray ionization conditions. High resolution mass spectra (HRMS) were obtained by a Waters Acquity UPLC coupled with a Waters Synapt G2 time-of-flight mass spec, using an ESI source. Compounds in DMSO solutions (5 mg/mL) were diluted to 0.01 mg/mL; 5 uL was injected on to a Waters BEH C18 1.7 μm 2.1 mm × 100 mm column equilibrated at 10% acetonitrile in water, spiked with 0.1% formic acid at a flow rate of 0.5 mL/min. Bound analytes were eluted by a linear gradient of 10−95% acetonitrile in water, between 1 and 8 min. The mass spectrum was calibrated using formate clusters; leucine enkephalin was used as a "lock mass" for additional, real-time, accurate-mass correction.

*Preparation of Indazole Intermediates. N1-Benzyl-6-bromo-1H-indazole (**11**).* A solution of 6-bromo-1*H*-indazole (**9**) (300 g, 1.52 mol) in dry DMSO (1.5 L) was treated with KO^{t}Bu (181 mL, 1.8 mol). After 10 min, BnCl (199 g, 1.8 mol) was added, and the resulting mixture left to stir at rt. After 4 h, the reaction was quenched with satd NH_4Cl (3 L) and extracted with MTBE (2 × 2 L). The combined organic phases were washed with brine (2 × 1 L) and concentrated to a red oil. The oil was slurried with heptane to give a yellow solid as a 3:1 mixture of *N*1- and *N*2-benzylated indazoles (**11** and **10**). The mixture was isomerized exclusively to the *N*1 isomer by dissolving into benzyl chloride (350 mL) and heating to 150 °C for 8 h. Excess benzyl chloride was distilled off at 150 °C to give a red oil which was treated with heptane (600 mL) to give **11** as a red solid (335 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 0.6 Hz, 1H), 7.58 (d, J = 8.6 Hz, 1H), 7.52 (s, 1H), 7.36−7.20 (m, 4H), 7.20−7.15 (m, 2H), 5.53 (s, 2H). LCMS (ESI) m/z calcd for $[C_{14}H_{11}BrN_2 + H]^+$ 287.0 (^{79}Br), 289.0 (^{81}Br); found, 287.0; 289.0.

*N1-Benzyl-6-vinyl-1H-indazole (**12**).* A mixture of **11** (300 g, 1.05 mol) and K_2CO_3 (432 g, 3.1 mol) in DME/water (3:1, 3.3 L) was purged with nitrogen. 4,4,5,5-Tetramethyl-2-vinyl-1,3,2-dioxaborolane (300 mL, 1.3 mol) was added, followed by $Pd(PPh_3)_2Cl_2$ (36 g, 5 mol %), and the mixture was heated to 80 °C for 7 h. The mixture was diluted with heptane (2 L) and washed with water (3 × 1 L) and brine (1 L). The organic phase was concentrated to 1 L and cooled to 0 °C. The resulting precipitate was collected and dried under high vacuum at 40 °C for 12 h to give the title compound as a yellow solid (160 g, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (s, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.29−7.19 (m, 5H), 7.18−7.13 (m, 2H), 6.75 (dd, J = 17.6, 10.9 Hz, 1H), 5.76 (d, J = 17.5 Hz, 1H), 5.53 (s, 2H), 5.26 (d, J = 10.9 Hz, 1H). LCMS (ESI) m/z calcd for $[C_{16}H_{14}N_2 + H]^+$ 235.1; found, 235.0.

*1-(N1-Benzyl-1H-indazol-6-yl)-ethane-1,2-diol (**13**).* Compound **12** (5.6 g, 24 mmol) was stirred in a mixture of *t*-BuOH (25 mL), water (25 mL), and citric acid (10.1 g, 48.0 mmol). *N*-Methylmorpholine-*N*-oxide (3.1 g, 26.4 mmol) and $K_2OsO_4{\cdot}2H_2O$ (44 mg, 0.12 mmol, 0.5 mol %) were added, and the resulting oily, biphasic mixture stirred overnight at rt. ^{1}H NMR of a sample showed approximately 40% conversion, and so the mixture was warmed to 50 °C (giving a single phase) for 7 h. Ethanol (50 mL) was added, and the mixture again stirred an additional 24 h at rt. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was taken up in 1.0 M aq HCl and EtOAc; the layers were separated and the aqueous phase extracted with further EtOAc. The combined organic portions were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was passed through a short silica plug, eluting with EtOAc, and concentrated to afford racemic **13** as a reddish gum (6.26 g, 97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.65 (d, J = 8.4 Hz, 1 H), 7.38 (s, 1H), 7.27−7.18 (m, 3H), 7.16−7.09 (m, 2H), 7.04 (d, J = 8.4 Hz, 1H), 5.50 (s, 2H), 4.86 (d, J = 7.6 Hz, 1H), 3.78−3.68 (m, 1H), 3.60 (t, J = 9.6 Hz, 1H), 3.46 (s, 1H), 2.84 (s, 1H). LCMS (ESI) m/z calcd for $[C_{16}H_{16}N_2O_2 + H]^+$, 269.1; found, 269.0.

*(S)-1-(N1-Benzyl-1H-indazol-6-yl)-ethane-1,2-diol (**13a**).* A 5 L clamp-top reaction vessel equipped with an overhead stirrer was treated with $K_3Fe(CN)_6$ (405 g, 1.23 mol), K_2CO_3 (170 g, 1.23 mol), $(DHQ)_2PHAL$ (3.2 g, 4.1 mmol), and $K_2OsO_4.2H_2O$ (1.54 g, 4.1 mmol). A mixture of *t*-BuOH and water (1:1, 4 L) was added, forming a clear, biphasic mixture on stirring. The mixture was cooled with an ice bath before addition of *N*1-benzyl-6-vinyl-1*H*-indazole (**12**) (96 g, 410 mmol). The resulting mixture was vigorously stirred at 0 °C for 4 h, and then warmed to 10 °C for 2 h. The reaction was quenched by portionwise addition of sodium metabisulfite (1 kg). The mixture was stirred for 1 h at rt and then filtered through a pad of Celite to remove precipitated OsO_2. The filtrate was extracted with CH_2Cl_2 (4 × 2 L), and the combined organic portions dried over $MgSO_4$, filtered, and concentrated. The crude product was recrystallized from hot toluene (10 mL/g) to afford the title compound as a white solid (74 g, 68%, 98% ee), with ^{1}H NMR and MS results identical to **13**. Chiral HPLC retention time, 16.8 min (Daicel Chiralpak IB (250 mm × 4.6 mm); isocratic 10% EtOH in *n*-heptane; 1 mL/min; ambient temperature.

*1-(1-Benzyl-1H-indazol-6-yl)ethane-1,2-diyl Dimethanesulfonate (**14**).* A solution of diol **13** (0.27 g, 1.0 mmol) and TEA (0.35 mL, 2.5 mmol) in dry DCM (5 mL) was cooled in an ice bath followed by dropwise addition of MsCl (0.15 mL, 2.0 mmol) over 5 min. The resulting mixture was left to stir for 2 h and warmed to rt. The mixture was diluted with DCM and washed quickly with 1.0 M aq HCl (20 mL), satd $NaHCO_3$ (40 mL), and brine (20 mL), dried over Na_2SO_4, filtered, and concentrated at 25 °C to afford the crude product. The crude product was purified by silica gel chromatography (1:1 Et_2O/CH_2Cl_2) to give **14** as an off-white, gummy foam (0.30 g, 71%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.81 (d, J = 8.3 Hz, 1H), 7.42 (s, 1H), 7.34−7.23 (m, 3H), 7.17 (dd, J = 18.1, 7.6 Hz, 3H), 5.89 (dd, J = 8.5, 3.2 Hz, 1H), 5.66 (d, J = 15.8 Hz, 1H), 5.60 (d, J = 15.8 Hz, 1H), 4.52 (dd, J = 11.8, 8.6 Hz, 1H), 4.40 (dd, J = 11.8, 3.2 Hz, 1H), 3.04 (s, 3H), 2.75 (s, 3H). LCMS (ESI) m/z calcd for $[C_{18}H_{20}N_2O_6S_2 + H]^+$, 425.1; found, 425.0.

*(S)-1-(1-Benzyl-1H-indazol-6-yl)ethane-1,2-diyl Dimethanesulfonate (**14a**).* A solution of **13a** (134 g, 0.5 mol) and TEA (174 mL, 1.25 mol) in CH_2Cl_2 (2.5 L) was cooled in an ice bath and treated by slow addition of MsCl (81 mL, 1.05 mol) over 1 h. The internal temperature increased to a maximum of 11 °C. The resulting mixture was left to stir for 30 min. The reaction was quenched with cold 1.0 M aq HCl (400 mL), the phases separated, and the organic phase washed with cold 1.0 M aq HCl, aq $NaHCO_3$, and brine, then dried over $MgSO_4$. The solution was poured onto a short silica gel pad under suction. The product was eluted using 1:1 Et_2O/CH_2Cl_2 (2 L). Removal of the solvents under reduced pressure gave a hard white solid, which was triturated with Et_2O (800 mL) overnight. The precipitate was collected by filtration and washed with further Et_2O (2 × 100 mL) to yield the title compound as a fine white powder (184 g, 87%, 99% ee) with ^{1}H NMR and MS results identical to **14**. Chiral HPLC retention time, 13.4 min (Daicel Chiralpak IB (250 mm × 4.6 mm); isocratic 30% EtOH in *n*-heptane; 1 mL/min; ambient temperature.

Cyclopropane Formation: General Method A. A solution of indolin-2-one (1 equiv) in dry THF (30 mL/g) was cooled in an ice bath. Sodium hydride (60 wt % in mineral oil, 2.1 equiv) was added in four portions, and the mixture was stirred at 0 °C for 15 min. A solution of 1 equiv of dimesylate (**14** or **14a**) in THF (30 mL/g) was added dropwise over 1 h and stirred for 2 h. The reaction was quenched with satd NH_4Cl solution, diluted with water, and extracted with EtOAc (3 volumes). The organic layer was dried with $MgSO_4$ and concentrated to a crude product, which was further purified by trituration or silica gel chromatography.

*rac-(1R,2S)-1′-Benzyl-2-(1-benzyl-1H-indazol-6-yl)spiro-[cyclopropane-1,3′-indolin]-2′-one (**15**).* Prepared according to general method A using oxindole **6** (45 mg, 0.2 mmol) and dimesylate **14** (85 mg, 0.2 mmol). The crude product was triturated with petroleum ether/EtOAc (3:1) and filtered to give the title compound **15** as a light-brown solid (75 mg, 82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (s, 1H), 7.60 (d, J = 8.3 Hz, 1H), 7.36−7.20 (m, 8H), 7.19 (s, 1H), 7.15−7.10 (m, J = 6.4 Hz, 2H), 6.99 (td, J = 7.8, 0.9 Hz, 1H), 6.92 (d, J = 8.3 Hz, 1H), 6.74 (d, J = 7.8 Hz, 1H), 6.50 (t, J = 7.4 Hz, 1H), 5.76 (d, J = 7.3 Hz, 1H), 5.61 (d, J = 15.8 Hz, 1H), 5.53 (d, J = 15.8 Hz, 1H), 5.08 (d, J = 15.6 Hz, 1H), 4.97 (d, J = 15.7 Hz, 1H), 3.48 (t, J = 8.5 Hz, 1H), 2.28 (dd, J = 9.0, 4.5 Hz, 1H), 2.02 (dd, J = 8.0, 4.6 Hz,

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

1H). LCMS (ESI) m/z calcd for $[C_{31}H_{25}N_3O + H]^+$, 456.2; found, 456.0.

*(1R,2S)-1′-Benzyl-2-(1-benzyl-1H-indazol-6-yl)spiro-[cyclopropane-1,3′-indolin]-2′-one (**15a**).* Prepared according to general method A using oxindole **6** (57 g, 224 mmol) and dimesylate **14a** (95 g, 224 mmol). The crude product was triturated with petroleum ether/EtOAc (3:1) and filtered to give the title compound **15a** as a light-brown solid (50 g, 46%, 98% ee) with ^{1}H NMR and MS results identical to **15**. Chiral HPLC retention time, 13.3 min (Daicel Chiralpak IA, 250 mm × 4.6 mm); isocratic 10% EtOH in *n*-heptane; 1 mL/min; ambient temperature.

*(1R,2S)-1′-Benzyl-2-(1-benzyl-1H-indazol-6-yl)-5′-methoxyspiro-[cyclopropane-1,3′-indolin]-2′-one (**23**).* Prepared according to general method A using dimethanesulfonate **14a** (95 g, 224 mmol) and 1-benzyl-5-methoxyindolin-2-one **19** (56 g, 224 mmol). The crude product was triturated with 3:1 petroleum ether/EtOAc to give **23** as a white solid (50 g, 46%, >99.9% ee, Chiralpak IA (250 mm × 4.6 mm); 1.0 mL/min; hexane/EtOH: 80/20). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H), 7.64 (d, J = 8.2 Hz, 1H), 7.36−7.23 (m, 9H), 7.14 (d, J = 7.2 Hz, 2H), 6.94 (d, J = 8.3 Hz, 1H), 6.63 (d, J = 8.3 Hz, 1H), 6.53 (d, J = 8.4 Hz, 1H), 5.61 (d, J = 15.1 Hz, 1H), 5.54 (d, J = 15.4 Hz, 1H), 5.37 (s, 1H), 5.07 (d, J = 15.4 Hz, 1H), 4.95 (d, J = 15.7 Hz, 1H), 3.51 (t, J = 8.1 Hz, 1H), 3.18 (s, 3H), 2.32−2.29 (m, 1H), 2.09−2.00 (m, 1H). LCMS (ESI) m/z calcd for $[C_{32}H_{27}N_3O_2 + H]^+$, 486.2; found, 486.3.

*(1R,2S)-2-(1-benzyl-1H-indazol-6-yl)-1′-methylspiro-[cyclopropane-1,3′-indolin]-2′-one (**24**).* Prepared according to the general method A using 1-methylindolin-2-one **20** (2.33 g, 15.8 mmol) and a solution of dimesylate **14a** (6.70 g, 15.8 mmol) in dry THF (45 mL). Purification via column chromatography (silica gel, 25−50% EtOAc in hexane) yielded the title compound as a pale-orange crystalline solid (5.0 g, 84%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (s, 1H), 7.60 (d, J = 8.3 Hz, 1H), 7.30−7.25 (m, 3H), 7.18 (s, 1H), 7.13−7.10 (m, 3H), 6.92 (d, J = 8.6 Hz, 1H), 6.85 (d, J = 7.6 Hz, 1H), 6.55 (t, J = 7.0 Hz, 1H), 5.76 (d, J = 7.2 Hz, 1H), 5.63−5.49 (m, 2H), 3.41 (t, J = 8.8 Hz, 1H), 3.33 (s, 3H), 2.22−2.18 (m, 1H), 2.00−1.96 (m, 1H); LCMS (ESI) m/z calcd for $[C_{25}H_{21}N_3O + H]^+$ 380.18; found, 380.2.

*(1R,2S)-2-(1-Benzyl-1H-indazol-6-yl)-5′-methoxy-1′-methylspiro-[cyclopropane-1,3′-indolin]-2′-one (**25**).* Prepared according to the general method A using dimethanesulfonate **14a** (1.44 g, 3.4 mmol) and 5-methoxy-1-methylindolin-2-one **21** (0.60 g, 3.4 mmol). Purification using Biotage Isolera (1−50% EtOAc in hexane, SNAP 25g column) yielded the title compound as a light-brown solid (1.05 g, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (d, J = 0.8 Hz, 1 H), 7.66 (d, J = 8.3 Hz, 1 H), 7.48 (s, 1 H), 7.17−7.31 (m, 3 H), 7.06−7.15 (m, 2 H), 6.92−6.99 (m, 1 H), 6.88 (d, J = 8.3 Hz, 1 H), 6.67 (dd, J = 8.5, 2.5 Hz, 1 H), 5.51−5.68 (m, 2 H), 5.41 (d, J = 2.5 Hz, 1 H), 3.33−3.40 (m, 1 H), 3.28 (s, 3 H), 3.15 (s, 3 H), 2.11−2.24 (m, 2 H). LCMS (ESI) m/z calcd for $[C_{26}H_{23}N_3O_2 + H]^+$, 410.2; found, 410.2.

*(1R,2S)-1′-Benzyl-2-(1-benzyl-1H-indazol-6-yl)-5′-methylspiro-[cyclopropane-1,3′-indolin]-2′-one (**26**).* The title compound was prepared according to the general method A using dimethanesulfonate **14a** (4.24 g, 10 mmol) and 1-benzyl-5-methylindolin-2-one **22** (2.37 g, 10 mmol). Purification by Biotage Isolera (20−30% EtOAc in hexane) gave **26** as a beige solid (3.2 g, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (s, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.36−7.20 (m, 9H), 7.14 (d, J = 6.0 Hz, 2H), 6.94 (d, J = 8.4 Hz, 1H), 6.80 (d, J = 8.0 Hz, 1H), 6.64 (d, J = 7.2 Hz, 1H), 5.62 (d, J = 16.8 Hz, 1H), 5.59 (s, 1H), 5.55 (d, J = 16.8 Hz, 1H), 5.08 (d, J = 16.0 Hz, 1H), 4.97 (d, J = 15.6 Hz, 1H), 3.48 (t, J = 8.4 Hz, 1H), 2.30−2.25 (m, 1H), 2.02−1.96 (m, 1H), 1.85 (s, 3H). LCMS (ESI) m/z calcd for $[C_{32}H_{27}N_3O + H]^+$, 470.2; found, 470.3.

Debenzylation: General Method B. A solution of benzylated indazol-6-ylspiro[cyclopropane-1,3′-indolin]-2′-ones (1 equiv) in THF (6 mL/g) was cooled in ice before addition of *t*-BuOK (1.7 M solution in THF, 20 equiv). DMSO (19 equiv) was added, and the mixture was purged gently with oxygen in an ice bath for 3 h and then warmed to rt overnight under an atmosphere of oxygen. Mixtures were generally quenched with 20% aq NH_4Cl, extracted with EtOAc, dried, and evaporated. The product was purified by silica gel chromatography or recrystallization.

*rac-(1R,2S)-2-(1H-Indazol-6-yl)spiro-[cyclopropane-1,3-indolin]-2′-one (**5**).* Prepared according to general method B using a solution of **15** (6.5 g, 14 mmol) in a mixture of DMSO (20 mL) and THF (200 mL). In this instance, the combined organic portions (4 × 50 mL EtOAc) were washed with satd aq sodium thiosulfate (50 mL) and brine (50 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was slurried in CH_2Cl_2 (100 mL) and poured onto a short silica pad (2 cm depth × 5 cm diameter) under suction. The major byproduct (R_f 0.6 in 1:1 EtOAc/cyclohexane) was eluted using CH_2Cl_2 (ca. 1 L). The product (R_f 0.25 in 1:1 EtOAc/cyclohexane) was eluted (2% then 5% MeOH/EtOAc) and concentrated to afford the title compound (2.5 g, 64%) as a pale-brown solid with a diastereomeric excess of 94%.

*(1R,2S)-2-(1H-Indazol-6-yl)spiro-[cyclopropane-1,3-indolin]-2′-one (**5a**).* Prepared according to general method B using a solution of **15a** (10 g, 22 mmol). The material was purified by dry-flash silica pad (4 cm depth × 20 cm diameter). Byproducts were eluted using solvent of increasing eluent strength (0:1, 1:9, 1:4 EtOAc/cyclohexane), and then the product was eluted using EtOAc to give the title compound as a beige solid (4.2 g, 70%, >98.5% ee). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.01 (s, 1H), 10.61 (d, J = 8.3 Hz, 1H), 8.01 (s, 1H), 7.63 (d, J = 8.3 Hz, 1H), 7.44 (s, 1H), 6.99 (t, J = 7.5 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 6.51 (t, J = 7.0 Hz, 1H), 5.98 (d, J = 8.0 Hz, 1H), 3.20−3.17 (m, 1H), 2.30−2.26 (m, 1H), 2.00−1.95 (m, 1H). LCMS (ESI) m/z calcd for $[C_{17}H_{13}N_3O + H]^+$, 276.3; found, 276.1; chiral HPLC retention time, 14.3 min; Daicel Chiralpak AS-H (250 mm × 4.6 mm); isocratic 40% EtOH in *n*-heptane; 1 mL/min; 35 °C.

The title compound was also obtained from racemic **5** (25 mg) by separation using chiral SFC, Chiralpak IA (3 cm × 15 cm), isocratic 30% MeOH (0.1% DEA)/CO_2, 70 mL/min) to give a white solid **5a** (11.5 mg, 97% ee, retention time, 5.2 min; Chiralpak IA (15 cm × 0.46 cm), 3.0 mL/min with isocratic 40% MeOH (0.1% DEA)/CO_2, 3.0 mL/min). From this resolution process, the opposite enantiomer, (1*S*,2*R*)-2-(1*H*-indazol-6-yl)spiro-[cyclopropane-1,3-indolin]-2′-one (**5b**) was isolated as a white solid (11.8 mg, >98% ee, retention time: 2.7 min) with ^{1}H NMR and LCMS as described for **5a**.

*(1R,2S)-2-(1H-Indazol-6-yl)-5′-methoxyspiro[cyclopropane-1,3′-indolin]-2′-one (**16a**).* The title compound was prepared according to the general method B using **23** (50 g, 103 mmol). Recrystallization from ethanol gave **16a** as a beige solid (22.3 g, 71%, 99.4% ee, Chiralpak IA (250 mm × 4.6 mm), 1.0 mL/min with isocratic 30% MeOH (0.1% DEA)/70% CO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.02 (br s, 1H), 10.42 (br s, 1H), 8.02 (s, 1H), 7.64 (d, J = 8.3 Hz, 1H), 7.45 (s, 1H), 6.94 (d, J = 7.8 Hz, 1H), 6.73 (d, J = 8.2 Hz, 1H), 6.55 (d, J = 8.6 Hz, 1H), 5.62 (s, 1H), 3.20 (s, 3H), 3.18 (t, J = 8.7 Hz, 1H), 2.34−2.28 (m, 1H), 1.98−1.95 (m, 1H). LCMS (ESI) m/z calcd for $[C_{18}H_{15}N_3O_2 + H]^+$, 306.1; found, 306.1. Optical rotation: $[\alpha]^{23}_D$ = −225° (*c* 0.44, MeOH).

*(1R,2S)-2-(1H-Indazol-6-yl)-1′-methylspiro[cyclopropane-1,3′-indolin]-2′-one (**27**).* The title compound was prepared according to the method B using **24** (1.16 g, 3.06 mmol). Purification via column chromatography (silica gel, 3−6% MeOH in CH_2Cl_2) yielded the title compound as a pale-yellow solid (656 mg, 74%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.06 (br. s, 1H), 8.05 (s, 1H), 7.64 (d, J = 7.6 Hz, 1H), 7.36 (s, 1H), 7.14 (t, J = 8.7 Hz, 1H), 6.97 (d, J = 8.7 Hz, 1H), 6.87 (d, J = 8.2 Hz, 1H), 6.62 (t, J = 7.6 Hz, 1H), 5.91 (d, J = 7.9 Hz, 1H), 3.46 (t, J = 7.8 Hz, 1H), 3.34 (s, 3H), 2.26−2.23 (m, 1H), 2.08−2.04 (m, 1H). LCMS (ESI) m/z calcd for $[C_{18}H_{15}N_3O + H]^+$, 290.13; found, 290.1.

*(1R,2S)-2-(1H-Indazol-6-yl)-5′-methoxy-1′-methylspiro-[cyclopropane-1,3′-indolin]-2′-one (**28**).* Prepared according to the general method B using **25** (0.875 g, 2.1 mmol). The resultant pale-yellow residue was purified by silica gel column chromatography using 5−10% EtOAc in hexane to give the title compound as an off-white solid (445 mg, 65%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (s, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.47 (s, 1H), 6.95−6.90 (m, 2H),

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

6.68 (d, J = 8.8 Hz, 1H), 5.58 (s, 1H), 3.38 (t, J = 8.4 Hz, 1H), 3.32 (s, 3H), 3.20 (s, 3H), 2.28 (dd, J = 9.2, 4.4 Hz, 1H), 2.06 (dd, J = 8.4, 4.8 Hz, 1H). LCMS (ESI) m/z calcd for $[C_{19}H_{17}N_3O_2 + H]^+$, 320.2; found, 320.1.

*(1R,2S)-2-(1H-Indazol-6-yl)-5′-methylspiro[cyclopropane-1,3′-indolin]-2′-one (**29**).* Prepared according to the general method B using **26** (1.4 g, 3 mmol). Purification by Biotage Isolera (10−95% EtOAc in hexane) gave the title compound as a light solid (680 mg, 53%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (s, 1H), 7.67 (d, J = 8.4 Hz, 1H), 7.46 (s, 1H), 6.94 (d, J = 8.4 Hz, 1H), 6.85 (d, J = 8.0 Hz, 1H), 6.81 (d, J = 7.6 Hz, 1H), 5.78 (s, 1H), 3.34−3.03 (m, 1H), 2.20−2.12 (m, 2H), 1.87 (s, 3H). LCMS (ESI) m/z calcd for $[C_{18}H_{15}N_3O + H]^+$, 290.1; found, 290.1.

Iodination: General Method C. To a solution of cyclopropylindolin-2-one (1 equiv) and K_2CO_3 (2 equiv) in DMF was added iodine (1.5 equiv). The resulting mixture was stirred for 18 h at rt and then poured with stirring into 1% aqueous $Na_2S_2O_3{\cdot}5H_2O$. The resulting solid was filtered and washed with water (2×) to give a crude product, which was further purified by trituration or silica gel chromatography.

*rac-(1R,2S)-2-(3-Iodo-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one (**17**).* Prepared according to general method C by treating a solution of **5** (200 mg, 0.72 mmol) and K_2CO_3 (300 mg, 2.15 mmol) in dry DMF (2 mL) with a solution of I_2 (500 mg, 1.97 mmol) in dry DMF (2 mL). The crude product was triturated with diethyl ether to give a beige solid (225 mg, 78%) identical to the material previously described.[24]

*(1R,2S)-2-(3-Iodo-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one (**17a**) and (1S,2R)-2-(3-Iodo-1H-indazol-6-yl)spiro-[cyclopropane-1,3′-indolin]-2′-one (**17b**).* The title compounds were obtained from racemic (1R*,2S*)-2-(3-iodo-1*H*-indazol-6-yl)-spiro[cyclopropane-1,3′-indolin]-2′-one (1 g) by separation using chiral SFC: Chiralcel OJ-H (2 cm × 25 cm), isocratic 40% methanol (0.1% DEA)/CO_2, 100 bar at 50 mL/min), to give a white solid **17a** (478 mg) with >99% ee and a retention time of 1.7 min (Chiralcel OJ-H (10 cm × 0.46 cm), isocratic 40% methanol (0.1% DEA)/CO_2; 3.0 mL/min). From this resolution process, the opposite enantiomer, **17b** was isolated as a white solid (426 mg, >99% ee, retention time: 2.5 min).

Compound **17a** was also obtained by direct preparation: **5a** (10.6 g, 38 mmol) was dissolved into dioxane (150 mL) and 2 M aq NaOH (150 mL). Iodine (13.4 g, 53.3 mmol) was added portionwise, and the reaction was stirred for 12 h. The reaction was quenched with 1% aqueous $Na_2S_2O_3{\cdot}5H_2O$ and extracted with EtOAc (3 × 150 mL). Purification by column chromatography on silica (20 cm depth × 4 cm diameter) using 1:1 EtOAc/cyclohexane gave the title compound **17a** (7.0 g, 46%, 98.5% ee) as an off-white powder. ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.47 (s, 1H), 10.62 (s, 1H), 7.47 (s, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.02−6.98 (m, 2H), 6.84 (d, J = 7.6 Hz, 1H), 6.53 (t, J = 7.6 Hz, 1H), 5.97 (d, J = 7.6 Hz, 1H), 3.18 (q, J = 8.4 Hz, 1H), 2.31 (dd, J = 7.2, 4.8 Hz, 1H), 1.98 (dd, J = 8.8, 4.8 Hz, 1H). LCMS (ESI) m/z calcd for $[C_{17}H_{12}IN_3O + H]^+$, 402.0; found, 402.0. Optical rotation: $[\alpha]^{22}_D$ = −118° (c 0.57, MeOH). Chiral HPLC retention time, 14.1 min; Daicel Chiralpak AS-H (250 mm × 4.6 mm); isocratic 40% EtOH in n-heptane; 1 mL/min; 35 °C.

*(1R,2S)-2-(3-Iodo-1H-indazol-6-yl)-5′-methoxyspiro-[cyclopropane-1,3′-indolin]-2′-one (**18a**).* The title compound was obtained from racemic (1R,2S)-2-(3-iodo-1*H*-indazol-6-yl)-5′-methoxyspiro[cyclopropane-1,3′-indolin]-2′-one[24] (15 g) by separation using chiral SFC: Chiralcel OJ-H (3 cm × 15 cm), (30% methanol (0.1% DEA)/CO_2, 75 mL/min), to give a white solid **18a** (6.75 g, 99% ee; chiral HPLC retention time, 2.1 min; Chiralpak IA (150 mm × 4.6 mm), 3.0 mL/min isocratic 40% 2-propanol (0.1% DEA)/CO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.48 (br s, 1H), 10.43 (br s, 1H), 7.49 (s, 1H), 7.31 (d, J = 8.6 Hz, 1H), 7.02 (d, J = 8.1 Hz, 1H), 6.73 (d, J = 8.4 Hz, 1H), 6.57 (d, J = 8.6 Hz, 1H), 5.62 (s, 1H), 3.29 (s, 3H), 3.19 (t, J = 8.4 Hz, 1H), 2.36−2.32 (m, 1H), 1.99−1.96 (m 1H). LCMS (ESI) m/z calcd for $[C_{18}H_{14}IN_3O_2 + H]^+$, 432.0; found, 432.1 $[M + 1]^+$. From this resolution process, the opposite enantiomer (1S,2R)-2-(3-iodo-1*H*-indazol-6-yl)-5′-methoxyspiro[cyclopropane-1,3′-indolin]-2′-one **18b** was isolated as a white solid (6.6 g, 99% ee, chiral HPLC retention time, 3.4 min) with ^{1}H NMR and LCMS as described for **18a**.

Alternatively, a solution of **16a** (250 g, 0.82 mol) in DMF (500 mL) and MeOH (500 mL) was treated with potassium carbonate (226 g, 1.64 mol). Iodine (340 g, 1.06 mol) was dissolved into DMF (475 mL) and added to the mixture. The mixture was stirred at rt for 6 h and quenched with sodium thiosulfate pentahydrate (360 g, 1.46 mol) in water (5.7 L). The mixture was stirred at rt for 4 h and filtered. The solid was washed with water (2 L) and dried under vacuum at 50 °C to give the title compound as a white solid (334 g, 95%, 98% ee) with ^{1}H NMR and LCMS as described above. Optical rotation: $[\alpha]^{22}_D$ = −143° (c 0.40, MeOH).

*(1R,2S)-2-(3-Iodo-1H-indazol-6-yl)-1′-methylspiro[cyclopropane-1,3′-indolin]-2′-one (**30**).* Prepared according to the general method C using **27** (930 mg, 3.21 mmol). Precipitation with EtOAc followed by filtration and rinsing with EtOAc gave the title compound (970 mg, 73%, >98% ee). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.96 (br. s, 1H), 7.43−7.39 (m, 2H), 7.16 (t, J = 7.6 Hz, 1H), 7.05 (d, J = 8.0 Hz, 1H), 6.89 (d, J = 7.8 Hz, 1H), 6.66 (t, J = 7.2 Hz, 1H), 5.91 (d, J = 8.0 Hz, 1H), 3.47 (t, J = 8.4 Hz, 1H), 3.35 (s, 3H), 2.30−2.26 (m, 1H), 2.08−2.04 (m, 1H). LCMS (ESI) m/z calcd for $[C_{18}H_{14}IN_3O + H]^+$, 416.03; found, 416.0. Optical rotation: $[\alpha]^{23}_D$ = −210° (c 0.4, MeOH). Chiral HPLC retention time, 2.4 min; Phenomenex Lux 5 μ Amylose-2 150 mm × 4.6 mm (2.5 mL/min with isocratic at 20% EtOH in hexane for 0.5 min, then gradient 20−50% EtOH in hexane over 2.5 min, then isocratic at 50% for 1 min).

*(1R,2S)-2-(3-Iodo-1H-indazol-6-yl)-5′-methoxy-1′-methylspiro-[cyclopropane-1,3′-indolin]-2′-one (**31**).* Prepared according to the general method C using **28** (1.34 g, 4.19 mmol). The title compound was isolated as a cream-colored solid (1.71 g, 91%, 97.9% ee). ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.38 (s, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.35 (s, 1H), 7.04 (d, J = 8.8 Hz, 1H), 6.77 (d, J = 8.4 Hz, 1H), 6.66 (d, J = 8.4 Hz, 1H), 5.53 (s, 1H), 3.46 (t, J = 8.0 Hz, 1H), 3.38 (s, 3H), 3.32 (s, 3H), 2.24 (dd, J = 8.4, 4.8 Hz, 1H), 2.04 (dd, J = 12.4, 4.8 Hz, 1H). LCMS (ESI) m/z calcd for $[C_{19}H_{16}IN_3O_2 + H]^+$, 446.0; found, 446.1 $[M + 1]^+$. Optical rotation: $[\alpha]^{22}_D$ = −134° (c 0.24, MeOH). Chiral HPLC retention time, 2.6 min; Phenomenex Lux 5μ Amylose-2 150 mm × 4.6 mm (2.5 mL/min with isocratic at 20% EtOH in hexane for 0.5 min, then gradient 20−50% EtOH in hexane over 2.5 min, then isocratic at 50% for 1 min).

*(1R,2S)-2-(3-Iodo-1H-indazol-6-yl)-5′-methylspiro[cyclopropane-1,3′-indolin]-2′-one (**32**).* Prepared according to the general method C using **29** (680 mg, 2.35 mmol). Purification by Biotage Isolera (EtOAc/hexane gradient: 10−90%) gave the title compound as a light-yellow solid (794 mg, 81%, >98% ee). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.43 (s, 1H), 10.51 (s, 1H), 7.47 (s, 1H), 7.32 (d, J = 8.4 Hz, 1H), 7.02 (d, J = 8.8 Hz, 1H), 6.81 (d, J = 8.4 Hz, 1H), 6.73 (d, J = 7.6 Hz, 1H), 5.86 (s, 1H), 3.18 (t, J = 8.2 Hz, 1H), 2.30−2.20 (m 1H), 2.00−1.90 (m, 1H), 1.85 (s, 3H). LCMS (ESI) m/z calcd for $[C_{18}H_{14}IN_3O + H]^+$, 416.0; found, 416.0. Optical rotation: $[\alpha]^{23}_D$ = −145° (c 0.49, MeOH). Chiral HPLC retention time, 9.6 min; Phenomenex Lux 5μ Cellulose-2 (150 mm × 4.6 mm); isocratic 10% EtOH in n-hexane (1.75 mL/min at ambient temperature).

*(1R,2S)-2-(1-Benzyl-3-iodo-1H-indazol-6-yl)-1′-methylspiro-[cyclopropane-1,3′-indolin]-2′-one (**33**).* To compound **30** (200 mg, 0.48 mmol) in DCM (3.5 mL) and 50% aq KOH (1 mL) was added benzyl bromide (69 μL, 0.58 mmol) and TBAB (2 mg, 0.005 mmol). The mixture was stirred overnight at 0 °C. The reaction was warmed to rt. DCM (10 mL) was added, and the mixture was washed with brine (2 × 5 mL), dried over $MgSO_4$, and concentrated. The residue was purified by Biotage silica gel chromatography (99:1 to 95:5 DCM/MeOH). The title compound was recrystallized from hot methanol to give a white crystalline solid (150 mg, 63%). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 7.35 (d, J = 8.3 Hz, 1H), 7.20−7.32 (m, 4H), 7.07−7.18 (m, 4H), 6.98 (d, J = 8.3 Hz, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.56 (t, J = 7.6 Hz, 1H), 5.72 (d, J = 7.55 Hz, 1H), 5.63- 5.49 (m, 2H), 3.39 (t, J = 8.5 Hz, 1H), 3.32 (s, 3H), 2.20 (dd, J = 4.6, 8.4 Hz, 1H), 1.87−1.99 (m, 1H). LCMS (ESI) m/z calcd for $[C_{25}H_{20}IN_3O + H]^+$, 506.1; found, 506.1. Single crystal X-ray crystallography structure determination was performed at the Service Crystallography Laboratory,

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

University of Toronto, Toronto, ON. The crystal and structure refinement data for compound **33** is given in the Supporting Information, and the corresponding ORTEP diagram is shown in Figure 1.

Suzuki–Miyaura Cross Coupling of 3-Iodospirocyclopropaneindolinones. *General Method D.* The appropriate 3-iodospirocyclopropaneindolinone (1 equiv) was dissolved into dioxane and 1 M aq Na_2CO_3 (5 equiv). The appropriate vinyl boronic ester (1.2 equiv), LiCl (3 equiv), and catalyst, $Pd(PPh_3)_4$ (2.5 mol %), were added and resulting mixture purged with argon. The mixture was heated on an oil bath for 18 h, cooled to rt, and diluted with satd $NaHCO_3$ and extracted with 3 volumes of EtOAc. The combined organic fractions were dried over $MgSO_4$ and concentrated. After removal of solvents, the residue was either purified by prep-HPLC to give the title compound as a TFA salt or by flash chromatography, followed by treatment with 1 M HCl in ether to generate the HCl salt.

General Method E. The appropriate 3-iodospirocyclopropaneindolinone (1 equiv) was dissolved into 2:1 toluene/ethanol and 1 M aq Na_2CO_3 (2 equiv). The appropriate vinyl boronic ester (1.2 equiv) and catalyst, $Pd(PPh_3)_4$ (5 mol %), were added, and the resulting mixture was heated in a microwave for 2 h at 125 °C. After cooling to rt, the mixture was diluted with H_2O, extracted with EtOAc (×2), and dried ($MgSO_4$). After removal of solvents, the residue was redissolved in MeOH and filtered through a Waters CX column and either purified by prep-HPLC to give the title compound as a TFA salt or by flash chromatography followed by treatment with 1 M HCl in ether to generate the HCl salt.

*(1R,2S)-(E)-2-(3-(4-(((Dimethylamino)methyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**3a**).* To a solution of **17a** (125 mg, 0.31 mmol) in DMF (1.6 mL) and water (0.4 mL) was added (*E*)-*N*,*N*-dimethyl-1-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)phenyl) methanamine (143 mg, 0.5 mmol), potassium fluoride (36 mg, 0.62 mmol), and $Pd(PPh_3)_4$ (18 mg, 0.015 mmol). The mixture was heated to 120 °C for 2 h under microwave irradiation. EtOAc (50 mL) was added, and the solution was washed with water (2 × 5 mL) and brine (5 mL) and dried over $MgSO_4$. Purification by reverse phase prep-HPLC gave the title compound as a yellow solid (73 mg, 44%). ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.02 (d, J = 8.3 Hz, 1H), 7.78 (d, J = 8.3 Hz, 2H), 7.56–7.48 (m, 5H), 7.08–7.04 (m, 2H), 6.94 (d, J = 8.3 Hz, 1H), 6.59 (t, J = 7.8 Hz, 1H), 6.00 (d, J = 8.0 Hz, 1H), 4.38 (s, 2H), 3.39–3.33 (m, 1H), 2.90 (s, 6H), 2.28–2.22 (m, 1H), 2.22–2.17 (m, 1H). HRMS (ESI) m/z calcd for $[C_{28}H_{26}N_4O + H]^+$, 435.2185; found, 435.2192. HPLC: 99.1% at 270 nM.

*(1S,2R)-(E)-2-(3-(4-(((Dimethylamino)methyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**3b**).* To a solution of **17b** (20 mg, 0.05 mmol) in DMF (0.4 mL) and water (0.1 mL) was added (*E*)-*N*,*N*-dimethyl-1-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)phenyl) methanamine (25 mg, 0.08 mmol), potassium fluoride (6 mg, 0.1 mmol), and $Pd(PPh_3)_4$ (3 mg, 0.002 mmol). The mixture was heated to 120 °C for 2 h under microwave irradiation. EtOAc (50 mL) was added, and the solution was washed with water (2 × 5 mL) and brine (5 mL) and dried over $MgSO_4$. Purification by reverse phase prep-HPLC gave the title compound as a yellow solid (4 mg, 15%) with ^{1}H NMR and MS results identical to **3a**. HPLC purity: 99.8% at 270 nM.

*(1R,2S)-5′-Methoxy-2-(3-(4-(morpholinomethyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one Hydrochloride (**4a**).* Prepared according to general method D using **18a** (5.3 g, 12.3 mmol) and (*E*)-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)morpholine (4.86 g, 14.8 mmol). The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 9:1) to give a solid, which was sonicated with Et_2O and filtered to give a white solid. The solid was dissolved into THF (50 mL), and then HCl (9 mL of a 1 M solution in Et_2O) was added. A precipitate formed immediately, and further precipitate was obtained on addition of more Et_2O (150 mL). The solid was quickly filtered and washed with Et_2O to give an off-white solid (3.2 g, 48%, 99% ee). ^{1}H NMR (400 MHz, CD_3OD) δ 8.04 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.2 Hz, 2H), 7.75–7.50 (m, 5H), 7.07 (d, J = 8.6 Hz, 1H), 6.83 (d, J = 8.5 Hz, 1H), 6.61 (dd, J = 8.5 Hz, 2.2 Hz, 1H), 5.58 (d, J = 2.2 Hz, 1H), 4.39 (s, 2H), 4.09–4.04 (m, 2H), 3.78–3.72 (m, 2H), 3.43–3.33 (m, 3H), 3.27–3.20 (m, 5H), 2.27–2.23 (m, 1H), 2.21–2.16 (m, 1H). HRMS (ESI) m/z calcd for $[C_{31}H_{30}N_4O_3 + H]^+$, 507.2396 $[M + 1]^+$; found, 507.2395. Optical rotation: $[\alpha]^{23}_D$ = −97° (c 0.5, MeOH). HPLC purity: 97.1% at 270 nM.

*(1S,2R)-5′-Methoxy-2-(3-((E)-4-(morpholinomethyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one Hydrochloride (**4b**).* Prepared according to the general method D using **18b** (509 mg, 1.18 mmol) and (*E*)-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)morpholine (468 mg, 1.42 mmol). Silica gel purification (93:7 to 92:8, CH_2Cl_2/MeOH) gave 374 mg of the free base, which was dissolved into THF (15 mL) and treated with HCl (0.9 mL of a 1 M solution in Et_2O), and the resulting precipitate was filtered and washed with Et_2O to give the title compound as an off-white powder (329 mg, 51%) with ^{1}H NMR and MS results identical to **4a**. Optical rotation: $[\alpha]^{24}_D$ = +102° (c 0.53, MeOH). HPLC purity: 99.2% at 270 nM.

*(1R,2S)-2-(3-((E)-2-(Pyridin-3-yl)vinyl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one (**34**).* Prepared according to general method D, using **17a** (61 mg, 0.147 mmol) and (*E*)-3-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)pyridine (50 mg, 0.2 mmol) (58 mg, 0.176 mmol). EtOAc (50 mL) was added, and the solution was washed with water (2 × 5 mL), brine (5 mL) and dried over $MgSO_4$. Purification by reverse phase prep-HPLC gave the title compound as a yellow solid (20 mg, 44%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.21 (s, 1H), 10.64 (s, 1H), 8.86 (s, 1H), 8.45 (d, J = 3.2 Hz, 1H), 8.15 (d, J = 8.0 Hz, 1H), 8.09 (d, J = 8.4 Hz, 1H), 7.65 (d, J = 16.8 Hz, 1H), 7.49 (d, J = 16.8 Hz, 1H), 7.47 (s, 1H), 7.40 (dd, J = 8.0 Hz, 4.8 Hz, 1H), 7.03 (d, J = 8.4 Hz, 1H), 6.99 (d, J = 7.2 Hz, 1H), 6.86 (d, J = 7.6 Hz, 1H), 6.53 (t, J = 7.4 Hz, 1H), 6.01 (d, J = 7.6 Hz, 1H), 3.21 (t, J = 8.4 Hz, 1H), 2.32 (dd, J = 7.6 Hz, 4.8 Hz, 1H), 2.00 (dd, J = 9.0 Hz, 4.8 Hz, 1H). MS (ESI) m/z calcd for $[C_{24}H_{18}N_4O + H]^+$, 379.1; found, 379.1. HPLC purity; 95% at 270 nM.

*(1R,2S)-5′-Methoxy-2-(3-((E)-2-(6-(4-methylpiperazin-1-yl)-pyridin-3-yl)vinyl)-1H-indazol-6-yl)-spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**35**).* Prepared according to the general method D using **18a** (130 mg, 0.30 mmol) with (*E*)-1-methyl-4-(5-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)pyridin-2-yl)piperazine (119 mg, 0.36 mmol). The reaction mixture was diluted with MeOH (12.5 mL) and filtered through a plug of silica (5 g), eluting with 2 M NH_3 in MeOH (100 mL). After removal of the solvents in vacuo, the title compound was purified by prep-HPLC to yield a yellow solid (104 mg, 34%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.26 (s, 1H), 8.00 (d, J = 8.4 Hz, 1H), 7.92 (d, J = 9.2 Hz, 1H), 7.45 (s, 1H), 7.40 (d, J = 16.8 Hz, 1H), 7.28 (d, J = 16.8 Hz, 1H), 7.03 (d, J = 7.6 Hz, 1H), 6.87 (d, J = 9.2 Hz, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.61 (dd, J = 8.4, 2.4 Hz, 1H), 5.58 (d, J = 2.0 Hz, 1H), 3.61 (m, 4H), 3.38 (m, 1H), 3.26 (s, 3H), 2.57 (m, 4H), 2.36 (s, 3H), 2.21 (m, 2H). HRMS (ESI) m/z calcd for $[C_{30}H_{30}N_6O_2 + H]^+$, 507.2508; found, 507.2497. HPLC: 99.9% at 270 nM.

*(1R,2S)-1′-Methyl-2-(3-((E)-2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)vinyl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one bis(2,2,2-trifluoroacetate) (**36**).* Prepared according to general method D, using **30** (61 mg, 0.147 mmol) and (*E*)-1-methyl-4-(5-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)pyridin-2-yl)-piperazine (58 mg, 0.176 mmol). Purification by prep-HPLC resulted in a pale-yellow solid, which was sonicated with Et_2O and filtered to give the title compound (21 mg, 20%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.35 (s, 1H), 8.05 (d, J = 8.4 Hz, 1H), 7.98 (d, J = 8.2 Hz, 1H), 7.44–7.33 (m, 3H), 7.14 (t, J = 7.5 Hz, 1H), 7.06–6.99 (m, 3H), 6.63 (t, J = 6.7 Hz, 1H), 6.02 (d, J = 7.7 Hz, 1H), 4.60–4.30 (m, 4H), 3.60–3.08 (m, 4H), 3.47–3.33 (m, 4H), 2.97 (s, 3H), 2.28–2.25 (m, 1H), 2.21–2.18 (m, 1H). HRMS (ESI) m/z calcd for $[C_{30}H_{30}N_6O + H]^+$, 491.2559; found, 491.2560. HPLC: 98.5% at 270 nM.

*(1R,2S)-(E)-2-(3-(4-(Piperazin-1-yl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**37**).* The title compound was synthesized according to the general method E using **17a** (40 mg, 0.1 mmol) and (*E*)-*tert*-butyl 4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)phenyl)piperazine-1-carboxylate (50 mg, 0.12 mmol). The crude Boc-protected amine from the

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX–XXX

reaction was dissolved into CH_2Cl_2 (10 mL), and TFA (1 mL) was added. The reaction was stirred for 3 h, the solvent was removed and the residue purified by prep-HPLC to yield the title compound (23 mg, 40%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.97 (d, J = 8.1 Hz, 1H), 7.57 (d, J = 8.6 Hz, 2H), 7.47−7.42 (m, 2H), 7.30 (d, J = 17 Hz, 1H), 7.06−7.01 (m, 4H), 6.93 (d, J = 7.6 Hz, 1H), 6.58 (t, J = 7.6 Hz, 1H), 5.98 (d, J = 8.0 Hz, 1H), 3.49−3.44 (m, 4H), 3.40−3.31 (m, 5H), 2.26−2.22 (m, 1H), 2.19−2.16 (m, 1H). HRMS (ESI) m/z calcd for $[C_{29}H_{27}N_5O + H]^+$, 462.2294; found, 462.2290. HPLC: 97.8% at 270 nM.

*(1R,2S)-(E)-2-(3-(4-((Dimethylamino)methyl)styryl)-1H-indazol-6-yl)-5′-methoxyspiro[cyclopropane-1,3′-indolin]-2′-one Hydrochloride (**38**).* The title compound was synthesized according to the general method E using **18a** (251 mg, 0.58 mmol) and (*E*)-*N*,*N*-dimethyl-1-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)-phenyl)methanamine (191 mg, 0.67 mmol). The product was extracted using EtOAc (40 mL) with a Varian 3 mL ChemElut cartridge. After removal of the solvents in vacuo, the title compound was purified by chromatography on Biotage (silica, SNAP-25g, 5−20% MeOH in DCM). Trituration with 1:1 Et_2O/DCM yielded the title compound (92 mg, 34%). HCl (1 M in Et_2O, 0.25 mL, 0.25 mmol) was added in a dropwise manner to an ice cooled solution of the free base (92 mg, 0.20 mmol) in THF (10 mL), and the resulting mixture was allowed to stir in ice for 40 min, then Et_2O (10 mL) was added to the mixture. Filtration under vacuum yielded the title compound as the hydrochloride salt (orange−red solid, 79 mg, 79%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 (d, J = 8.5 Hz, 1H), 7.77 (d, J = 8.0 Hz, 2H), 7.48−7.63 (m, 5H), 7.09 (d, J = 8.3 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.61 (dd, J = 8.3, 2.3 Hz, 1H), 5.61 (d, J = 2.3 Hz, 1H), 4.36 (s, 2 H), 3.35 (m, 1H), 3.28 (s, 3H), 2.89 (s, 6H), 2.26 (dd, J = 7.7, 5.1 Hz, 1H), 2.18 (dd, J = 8.8, 4.8 Hz, 1H). HRMS (ESI) m/z calcd for $[C_{29}H_{28}N_4O_2 + H]^+$, 465.2291; found, 465.2288. Optical rotation: $[\alpha]^{24}_D$ = −70° (c 0.44, MeOH). HPLC: 99.8% at 270 nM.

*(1R,2S)-(E)-2-(3-(4-((Dimethylamino)methyl)styryl)-1H-indazol-6-yl)-1′-methylspiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**39**).* Prepared according to the general method D using **30** (134 mg, 0.32 mmol) and (*E*)-*N*,*N*-dimethyl-1-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)phenyl)methanamine (111 mg, 0.39 mmol). Purification by prep-HPLC resulted in a pale-yellow solid, which was triturated with Et_2O and filtered to give the title compound (42 mg, 23%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (d, J = 8.0 Hz, 1H), 7.76 (d, J = 7.6 Hz, 2H), 7.54−7.47 (m, 5H), 7.15 (t, J = 8.2 Hz, 1H), 7.03 (d, J = 7.8 Hz, 2H), 6.64 (t, J = 7.5 Hz, 1H), 6.02 (d, J = 7.0 Hz, 1H), 4.33 (s, 2H), 3.41−3.35 (m, 4H), 2.88 (s, 6H), 2.29−2.26 (m, 1H), 2.22−2.19 (m, 1H). HRMS (ESI) m/z calcd for $[C_{29}H_{28}N_4O + H]^+$, 449.2341; found, 449.2336. Optical rotation: $[\alpha]^{22}_D$ = −152° (c 0.42, MeOH). HPLC: 96.8% at 270 nM.

*(1R,2S)-(E)-2-(3-(4-(Pyrolidin-1-ylmethyl)styryl)-1H-indazol-6-yl)-spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**40**).* The title compound was synthesized according to the general method E, using **17a** (600 mg, 1.5 mmol) and (*E*)-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)pyrrolidine (460 mg, 1.5 mmol). Purification by reverse phase prep-HPLC gave the title compound as a yellow solid (337 mg, 45%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (d, J = 8.6 Hz, 1H), 7.76 (d, J = 8.6 Hz, 2H), 7.55−7.48 (m, 5H), 7.08−7.05 (m, 2H), 6.94 (d, J = 7.8 Hz, 1H), 6.59 (t, J = 7.5 Hz, 1H), 5.99 (d, J = 7.5 Hz, 1H) 4.40 (s, 2H), 3.55−3.46 (m, 2H), 3.38−3.34 (m, 1H), 3.27−3.16 (m, 2H), 2.27−2.17 (m, 4H), 2.06−1.98 (m, 2H). HRMS (ESI) m/z calcd for $[C_{30}H_{28}N_4O + H]^+$, 461.2341; found, 461.2336. Optical rotation: $[\alpha]^{24}_D$ = −117° (c 0.52, MeOH). HPLC purity: 98.8% at 270 nM.

*(1R,2S)-(E)-2-(3-(4-(((R)-3-Fluoropyrrolidin-1-yl)methyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**41**).* The title compound was synthesized according to the general method D, using **17a** (630 mg, 1.57 mmol) and (3*R*)-3-fluoro-1-(4-((*E*)-2-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl)vinyl)-benzyl)pyrrolidine (622 mg, 1.88 mmol). The title product was obtained as a pale-yellow solid after prep-HPLC purification (338 mg, 48%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (d, J = 8.3 Hz, 1H), 7.73 (d, J = 7.8 Hz, 2H), 7.55−7.45 (m, 5H), 7.06−7.00 (m, 2H), 6.93 (d, J = 7.7 Hz, 1H), 6.56 (t, J = 7.5 Hz, 1H), 5.97 (d, J = 7.5 Hz, 1H), 5.46 (d, J = 52.4 Hz, 1H), 4.46 (s, 2H), 3.73−3.31 (m, 5H), 2.65−2.44 (m, 2H), 2.26−2.15 (m, 2H). HRMS (ESI) m/z calcd for $[C_{30}H_{27}FN_4O + H]^+$, 479.2247; found, 479.2242. Optical rotation: $[\alpha]^{22}_D$ = −125° (c 0.44, MeOH). HPLC: 99.2% at 270 nM.

*(1R,2S)-(E)-2-(3-(4-(Piperidin-1-ylmethyl)styryl)-1H-indazol-6-yl)-spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**42**).* The title compound was synthesized according to the general method E using **17a** (175 mg, 0.4 mmol) and (*E*)-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)piperidine (225 mg, 0.67 mmol). Purification by reverse phase prep-HPLC gave the title compound as a yellow TFA salt (140 mg, 60%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (d, J = 8.8 Hz, 1H), 7.77 (d, J = 8.6 Hz, 2H), 7.75−7.48 (m, 5H), 7.08−7.05 (m, 2H), 6.94 (d, J = 7.5 Hz, 1H), 6.59 (t, J = 7.7 Hz, 1H), 5.99 (d, J = 7.3 Hz, 1H) 4.31 (s, 2H), 3.53−3.45 (m, 2H), 3.39−3.34 (m, 1H), 3.04−2.93 (m, 2H) 2.27−2.17 (m, 2H), 2.02−1.95 (m, 2H), 1.88−1.71 (m, 3H), 1.57−1.45 (m, 1H). HRMS (ESI) m/z calcd for $[C_{31}H_{30}N_4O + H]^+$, 475.2498; found, 475.2501. Optical rotation: $[\alpha]^{23}_D$ = −109° (c 0.35, MeOH). HPLC: 98.2% at 270 nM.

*(1R,2S)-(E)-5′-Methoxy-2-(3-(4-(piperidin-1-ylmethyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**43**).* The title compound was synthesized according to the general method E using **18a** (175 mg, 0.4 mmol) and (*E*)-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)piperidine (225 mg, 0.67 mmol). Purification by reverse phase prep-HPLC gave a yellow powder (90 mg, 36%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (d, J = 8.3 Hz, 1H), 7.75 (d, J = 8.6 Hz, 2H), 7.53−7.49 (m, 5H), 7.05 (d, J = 8.5 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.61 (dd, J = 8.4, 2.3 Hz, 1H), 5.58 (d, J = 2.3 Hz, 1H), 4.30 (s, 2H), 3.52−3.44 (m, 2H), 3.38−3.34 (m, 1H), 3.26 (s, 3H), 3.01−2.93 (m, 2H), 2.26−2.17 (m, 4H), 2.00−1.91 (m, 2H), 1.89−1.67 (m, 3H), 1.58−1.46 (m, 1H). HRMS (ESI) m/z calcd for $[C_{32}H_{32}N_4O_2 + H]^+$, 505.2604; found, 505.2602. Optical rotation: $[\alpha]^{23}_D$ = −69° (c 0.29, MeOH). HPLC: 99.9% at 270 nM.

*(1R,2S)-(E)-2-(3-(4-(Morpholinomethyl)styryl)-1H-indazol-6-yl)-spiro[cyclopropane-1,3′-indolin]-2′-one Hydrochloride (**44**).* The title compound prepared according to the general method E, using iodide **17a** (2.5 g, 6.3 mmol) and (*E*)-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)morpholine (2.5 g, 7.5 mmol). Purification by Biotage silica gel chromatography (gradient 0−10% MeOH in CH_2Cl_2) gave the free base, which was dissolved into THF (50 mL) and treated with 1 M HCl in ether (6 mL) and diluted with diethyl ether (200 mL). The resulting precipitate was collected by filtration to give the title compound as a yellow solid (2.6 g, 79%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.03 (d, J = 8.3 Hz, 1H), 7.78 (d, J = 7.5 Hz, 2H), 7.49−7.61 (m, 5H), 7.05 (t, J = 8.3 Hz, 2H), 6.94 (d, J = 8.3 Hz, 1H), 6.58 (t, J = 7.4 Hz, 1H), 5.99 (d, J = 7.0 Hz, 1H), 4.40 (s, 2H), 4.08−4.05 (m, 2H), 3.80−3.74 (m, 2H), 3.43−3.36 (m, 3H), 3.27−3.21 (m, 2H), 2.26−2.18 (m, 2H). HRMS (ESI) m/z calcd for $[C_{30}H_{28}N_4O_2 + H]^+$, 477.2291; found, 477.2284. Optical rotation $[\alpha]^{24}_D$ = −79° (c 0.33, MeOH). HPLC: 95.1% at 270 nM.

*(1R,2S)-(E)-5′-Methyl-2-(3-(4-(morpholinomethyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**45**).* Prepared according to the general method E using **32** (415 mg, 1 mmol) and (*E*)-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)morpholine (329 mg, 1 mmol). Purification by prep-HPLC gave title compound as a yellow solid (470 mg, 78%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.73 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 8.0 Hz, 2H), 7.44 (d, J = 8.0 Hz, 2H), 7.38 (s, 1H), 7.32 (d, J = 16.8 Hz, 1H), 7.27 (d, J = 16.8 Hz, 1H), 6.82 (d, J = 8.4 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 6.73 (d, J = 8.0 Hz, 1H), 5.78 (s, 1H), 4.29 (s, 2H), 3.99 (d, J = 11.2 Hz, 2H), 3.75 (t, J = 11.6 Hz, 2H), 3.42−3.32 (m, 2H), 3.21 (t, J = 8.4 Hz, 1H), 3.18−3.08 (m, 2H), 2.09−2.01 (m, 2H), 1.72 (s, 3H). HRMS (ESI) m/z calcd for $[C_{31}H_{30}N_4O_2 + H]^+$, 491.2447; found, 491.2445. Optical rotation $[\alpha]^{23}_D$ = −89° (c 0.28, MeOH). HPLC: 99.5% at 270 nM.

*(1R,2S)-(E)-5′-Methoxy-1′-methyl-2-(3-(4-(morpholinomethyl)-styryl)-1H-indazol-6-yl)spiro-[cyclopropane-1,3′-indolin]-2′-one Hydrochloride (**46**).* The title compound was synthesized according to

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

the general method D using **31** (5 g, 11.2 mmol) and (*E*)-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)morpholine (4.8 g, 14.6 mmol). Purification by column chromatography (silica gel, CH_2Cl_2/MeOH, 95:5 to 92:8) gave the free base as a yellow solid, which was dissolved into THF (100 mL) and treated with (11.2 mL of a 1 M solution in Et_2O) which gave after drying, the title product as a pale-yellow solid (3.9 g, 67%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (d, J = 8.6 Hz, 1H), 7.77 (d, J = 8.0 Hz, 2H), 7.56–7.49 (m, 5H), 7.04 (d, J = 8.4 Hz, 1H), 6.92 (d, J = 7.8 Hz, 1H), 6.69 (d, J = 8.6 Hz, 1H), 5.63 (s, 1H), 4.39 (s, 2H), 4.08–4.05 (m, 2H), 3.77–3.71 (m, 2H), 3.35–3.23 (m, 11H), 2.27–2.25 (m, 1H), 2.22–2.19 (m, 1H). HRMS (ESI) m/z calcd for $[C_{32}H_{32}N_4O_3 + H]^+$, 521.2553; found, 521.2551. Optical rotation: $[\alpha]^{22}_D$ = −85° (c 0.59, MeOH). HPLC: 97.0% at 270 nM. Anal. ($C_{32}H_{36}ClN_4O_3 \cdot 1.5H_2O$) C, H, N.

*(1R,2S)-(E)-2-(3-(4-cis-2,6-Dimethylmorpholino)methyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one Hydrochloride (**47**).* Prepared according to the general method E using **17a** (8 g, 20 mmol) and *cis*-2,6-dimethyl-4-(4-((*E*)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)morpholine (8.4 g, 24 mmol). The residue was purified by Biotage silica gel chromatography using a gradient of 0–10% methanol in DCM. The free base was dissolved in THF (100 mL) and treated with 1 M HCl in ether (12 mL). Diethyl ether (200 mL) was added, and the precipitate was collected by filtration to give a yellow solid (5.6 g, 52%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.03 (d, J = 8.3 Hz, 1H), 7.78 (d, J = 8.3 Hz, 2H), 7.49–7.64 (m, 5H), 7.00–7.10 (m, 2H), 6.94 (d, J = 7.8 Hz, 1H), 6.58 (t, J = 7.5 Hz, 1H), 5.99 (d, J = 7.5 Hz, 1H), 4.37 (s, 2H), 3.81–3.95 (m, 2H), 3.34–3.41 (m, 3H), 2.79 (t, J = 11.8 Hz, 2H), 2.14–2.29 (m, 2H), 1.24 (d, J = 6.3 Hz, 6H). HRMS (ESI) m/z calcd for $[C_{32}H_{32}N_4O_2 + H]^+$, 505.2604; found, 505.2601. Optical rotation $[\alpha]^{23}_D$ = −134° (c 0.27, MeOH). HPLC: 99.9% at 270 nM. Anal. ($C_{32}H_{33}ClN_4O_2 \cdot 2.5H_2O$) C, H, N.

*(1R,2S)-(E)-2-(3-(4-cis-2,6-Dimethylmorpholino)methyl)styryl)-1H-indazol-6-yl)-5′-methoxyspiro[cyclopropane-1,3′-indolin]-2′-one Fumarate (**48**).* To a solution of **18a** (20 g, 46 mmol) and (*E*)-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)-benzyl)morpholine (18.2 g, 51 mmol) in dioxane (400 mL) and water (80 mL) was added Na_2CO_3 (9.8 g, 92.8 mmol) and $Pd(PPh_3)_4$ (1.61 g, 1.39 mmol). The mixture was heated to 85 °C for 24 h, cooled to rt, and diluted with water (320 mL). The mixture was concentrated to a volume of 450 mL and EtOAc (320 mL) was added and the organic layer was separated and washed with water (80 mL). The solution was concentrated and redissolved into EtOAc (300 mL) and treated with charcoal (3 g) at 40 °C for 1 h. The charcoal was filtered off and TMT (2.8 g) was added and the mixture stirred at 40 °C for 16 h. The TMT was filtered off, and the organic phase was washed with 0.5 N aq NaOH (3 × 120 mL) and brine (120 mL). The solvents were removed in vacuo, and the resulting residue was recrystallized from methyl acetate (65 mL) to give the free base as a white solid (11.2 g, 48%). The free base (9.0 g, 16.8 mmol) was dissolved into acetone (36 mL) and heated to 50 °C. In a separate flask, fumaric acid (1.94 g, 16.8 mmol) was dissolved into acetone (120 mL), heated to 50 °C, and added to the free base solution. The solution was concentrated to a volume of 50 mL, and MTBE was added (25 mL). The solution was cooled to rt and stirred 24 h. The filtrate was collected and dried under high vacuum at 70 °C to give the title compound as a white crystalline solid (10.1 g, 92%, 99.9% ee). ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (d, J = 8.4 Hz, 1H), 7.68 (d, J = 8.0 Hz, 2H), 7.50–7.45 (m, 5H), 7.03 (d, J = 8.1 Hz, 1H), 6.83 (d, J = 8.4 Hz, 1H), 6.73 (s, 2H), 6.60 (dd, J = 8.4, 2.5 Hz, 1H), 5.58 (s, 1H), 4.00 (s, 2H), 3.82–3.78 (m, 2H), 3.36 (t, J = 8.4 Hz, 1H), 3.26 (s, 3H), 3.13–3.10 (m, 2H), 2.34 (t, J = 11.4 Hz, 2H), 2.25–2.16 (m, 2H), 1.01 (d, J = 6.0 Hz, 6H). HRMS (ESI) m/z calcd for $[C_{33}H_{34}N_4O_3 + H]^+$, 535.2709; found, 535.2712. Optical rotation $[\alpha]^{20}_D$ = −97° (c 0.5, MeOH). HPLC: 99.7% at 270 nm. Anal. ($C_{37}H_{38}N_4O_7$) C, H, N.

*(1R,2S)-2-(3-((E)-(4-cis-2,6-Dimethylmorpholino)methyl)styryl)-1H-indazol-6-yl)-5′-methoxy-1′-methylspiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**49**).* The title compound was synthesized according to the general method E, using **31** (100 mg, 0.23 mmol) and (*E*)-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)morpholine (100 mg, 0.26 mmol). Purification by reverse phase prep-HPLC gave the title compound as a yellow solid (72 mg, 49%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.93 (d, J = 8.5 Hz, 1H), 7.71 (d, J = 7.8 Hz, 2H), 7.52 (d, J = 7.8 Hz, 2H), 7.49–7.45 (m, 3H), 6.95 (d, J = 8.5 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 6.66 (d, J = 8.5 Hz, 1H), 5.62 (s, 1H), 4.35 (s, 2H), 3.90–3.82 (s, 2H), 3.34–3.43 (m, 2H), 3.30 (s, 3H), 3.26 (s, 3H), 2.76 (t, J = 11.5 Hz, 2H), 2.13–2.28 (m, 2H), 1.23 (d, J = 6.0 Hz, 6H). HRMS (ESI) m/z calcd for $[C_{34}H_{36}N_4O_3 + H]^+$, 549.2866; found, 549.2855. Optical rotation: $[\alpha]^{23}_D$ = −69° (c 0.41, MeOH). HPLC: 98.2% at 270 nM.

*(1R,2S)-(E)-2-(3-(3-(Morpholinomethyl)styryl)-1H-indazol-6-yl)-spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**50**).* The title compound was synthesized according to the general method E using **17a** (160 mg, 0.4 mmol) and (*E*)-4-(3-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)morpholine (184 mg, 0.56 mmol). Purification by prep-HPLC gave the title compound as a yellow solid (158 mg, 67%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.88 (d, J = 8.4 Hz, 1H), 7.73 (s, 1H), 7.67 (d, J = 8.0 Hz, 1H), 7.50–7.38 (m, 5H), 6.98 (t, J = 7.8 Hz, 1H), 6.91 (d, J = 8.0 Hz, 2H), 6.48 (t, J = 7.6 Hz, 1H), 5.92 (d, J = 7.2 Hz, 1H), 4.36 (s, 2H), 4.03 (d, J = 11.6 Hz, 2H), 3.76 (t, J = 12.0 Hz, 2H), 3.45–3.14 (m, 5H), 2.17–2.08 (m, 2H). HRMS (ESI) m/z calcd for $[C_{30}H_{28}N_4O_2 + H]^+$, 477.2291; found, 477.2284. Optical rotation $[\alpha]^{23}_D$ = −144° (c 0.34, MeOH). HPLC: 97.7% at 270 nM.

*(1R,2S)-2-(3-((E)-3-((cis-2,6-Dimethylmorpholino)methyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**51**).* The title compound was synthesized according to the general method E using **17a** and (*cis*)-2,6-dimethyl-4-(3-((*E*)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)morpholine (105 mg, 0.3 mmol). Purification by reverse phase prep-HPLC gave the title compound as a yellow TFA salt (65 mg, 41%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.95 (d, J = 8.5 Hz, 1H), 7.67–7.81 (m, 2H), 7.36–7.57 (m, 5H), 6.88–7.08 (m, 3H), 6.53 (t, J = 7.6 Hz, 1H), 5.96 (d, J = 7.5 Hz, 1H), 4.37 (s, 2H), 3.87 (s, 2H), 3.34–3.45 (m, 3H), 2.81–2.75 (m, 2H), 2.05–2.26 (m, 2H), 1.22 (d, J = 6.0 Hz, 6H). HRMS (ESI) m/z calcd for $[C_{32}H_{32}N_4O_2 + H]^+$, 505.2604; found, 505.2600. Optical rotation: $[\alpha]^{23}_D$ = −128° (c 0.5, MeOH). HPLC: 99.5% at 270 nM.

*(1R,2S)-2-(3-((E)-4-((S)-1-Morpholinoethyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one Hydrochloride (**52**).* The title compound was synthesized according to the general method D, using **17a** (244 mg, 0.61 mmol) and 4-((1*S*)-1-(4-((*E*)-2-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl)vinyl)phenyl)ethyl)morpholine (230 mg, 0.67 mmol). Silica gel purification (94:6 to 95:5, CH_2Cl_2/MeOH) gave 105 mg, 35% of the free base which was dissolved into THF (10 mL) and treated with HCl (0.5 mL of a 1 M solution in Et_2O), and the resulting precipitate was filtered and washed with Et_2O to give the title compound as an off-white powder (87 mg, 29%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.01 (d, J = 8.4 Hz, 1H), 7.78 (d, J = 8.3 Hz, 2H), 7.56–7.47 (m, 5H), 7.07–7.03 (m, 2H), 6.93 (d, J = 7.5 Hz, 1H), 6.57 (t, J = 7.6 Hz, 1H), 5.98 (d, J = 7.7 Hz, 1H), 4.50 (q, J = 6.9 Hz, 1H), 4.12–4.09 (m, 1H), 4.00–3.96 (m, 1H), 3.87–3.81 (m, 1H), 3.73–3.67 (m, 2H), 3.35 (t, J = 8.2 Hz, 1H), 3.20–3.03 (m, 3H), 2.26–2.22 (m, 1H), 2.20–2.17 (m, 1H), 1.79 (d, J = 6.9 Hz, 3H). HRMS (ESI) m/z calcd for $[C_{31}H_{30}N_4O_2 + H]^+$, 491.2447; found, 491.2446. Optical rotation: $[\alpha]^{23}_D$ = −183° (c 0.58, MeOH). HPLC: 99.1% at 270 nM.

*(1R,2S)-2-(3-((E)-4-(8-Oxa-3-azabicyclo[3.2.1]octan-3-ylmethyl)-styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one Hydrochloride (**53**).* The title compound was synthesized according to the general method D using **17a** (250 mg, 0.62 mmol) and (*E*)-3-(4-(2-(4,4,6-trimethyl-1,3,2-dioxaborinan-2-yl)vinyl)benzyl)-8-oxa-3-azabicyclo[3.2.1]octane (332 mg, 0.93 mmol). Silica gel purification (96:4 to 95:5, CH_2Cl_2/MeOH) gave a crude material which was triturated with 4:1 hexanes/EtOAc to give 110 mg of the free base which was then dissolved into THF (2 mL) and treated with HCl (0.3 mL, 1 M in Et_2O), and the resulting precipitate was filtered and washed with Et_2O to give the title compound as an off-white powder (97 mg, 31%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.01 (d, J = 8.7 Hz,

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX–XXX

1H), 7.76 (d, J = 8.2 Hz, 2H), 7.58−7.48 (m, 5H), 7.07−7.03 (m, 2H), 6.93 (d, J = 7.8 Hz, 1H), 6.58 (t, J = 7.5 H, 1H), 5.98 (d, J = 7.5 Hz, 1H), 4.53 (bs, 2H), 4.35 (s, 2H), 3.36 (t, J = 8.8 Hz, 1H), 3.30−3.25 (m, 4H), 2.26−2.23 (m, 1H), 2.20−2.17 (m, 1H), 2.14−1.87 (m, 4H). HRMS (ESI) m/z calcd for $[C_{32}H_{30}N_4O_2 + H]^+$, 503.2447; found, 503.2443. Optical rotation: $[\alpha]^{23}_D = -174°$ (c 0.54, MeOH). HPLC: 99.6% at 270 nM.

*(1R,2S)-(E)-2-(3-(4-((1,4-Oxazepan-4-yl)methyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate Salt (**54**).* The title compound was synthesized according to the general method E, using **17a** (100 mg, 0.25 mmol) and (*E*)-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)-1,4-oxazepane (103 mg, 0.30 mmol). Purification by reverse phase prep-HPLC gave the title compound as a yellow powder (38 mg, 26%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.02 (d, J = 9.0 Hz, 1H), 7.78 (d, J = 8.3 Hz, 2H), 7.61−7.53 (m, 4H), 7.48 (s, 1H), 7.08−7.04 (m, 2H), 6.94 (d, J = 7.5 Hz, 1H), 6.60−6.56 (m, 1H), 5.99 (d, J = 7.8 Hz, 1H), 4.45 (s, 2H), 4.02−3.80 (m, 4H), 3.65−3.60 (m, 1H), 3.57−3.35 (m, 4H), 2.29−2.12 (m, 4H). HRMS (ESI) m/z calcd for $[C_{31}H_{30}N_4O_2 + H]^+$, 491.2447; found, 491.2440. Optical rotation $[\alpha]^{23}_D = -146°$ (c 0.39, MeOH). HPLC: 95.1% at 270 nM.

*(1R,2S)-2-(3-((E)-4-(((3S,5R)-3,4,5-Trimethylpiperazin-1-yl)methyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**55**).* The title compound was synthesized according to the general method E using **17a** (600 mg, 1.5 mmol) and (2*S*,6*R*)-1,2,6-trimethyl-4-(4-((*E*)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)benzyl)piperazine (618 mg, 1.8 mmol). Purification by reverse phase prep-HPLC gave the title compound as a yellow powder (296 mg, 31%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.97 (d, J = 8.5 Hz, 1H), 7.67 (d, J = 7.8 Hz, 2H), 7.51−7.41 (m, 5H), 7.08−6.98 (m, 2H), 6.94 (d, J = 8.0 Hz, 1H), 6.56 (t, J = 7.5 Hz, 1H), 5.98 (d, J = 7.3 Hz, 1H), 4.00 (s, 2H), 3.62−3.57 (m, 2H), 3.41−3.33 (m, 3H), 2.92 (s, 3H), 2.79 (t, J = 12.3 Hz, 2H), 2.26−2.12 (m, 2H), 1.41 (d, J = 6.3 Hz, 6H). HRMS (ESI) m/z calcd for $[C_{33}H_{35}N_5O + H]^+$, 518.2920; found, 518.2920. Optical rotation: $[\alpha]^{23}_D = -113°$ (c 0.37, MeOH). HPLC: 96.3% at 270 nM.

*(1R,2S)-5′-Methoxy-2-(3-((E)-4-(((2R,6S)-2,4,6-trimethylpiperazin-1-yl)methyl)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**56**).* The title compound was synthesized according to the general method E using **18a** (60 mg, 0.14 mmol) and (2*R*,6*S*)-2,4,6-trimethyl-1-(4-((*E*)-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)-benzyl)piperazine (60 mg, 0.16 mmol). Purification by reverse phase prep-HPLC gave the title compound as a yellow powder (35 mg, 46%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (d, J = 8.5 Hz, 1H), 7.58−7.67 (m, J = 8.0 Hz, 2H), 7.44−7.50 (m, 3H), 7.36−7.44 (m, 2H), 7.03 (d, J = 7.5 Hz, 1H), 6.83 (d, J = 8.5 Hz, 1H), 6.61 (dd, J = 8.4, 2.4 Hz, 1H), 5.59 (d, J = 2.5 Hz, 1H), 3.75 (s, 2H), 3.44 (bs, 2H), 3.36 (t, J = 8.5 Hz, 1H), 3.26 (s, 3H), 3.17−3.14 (m, 2H), 2.90 (s, 3H), 2.46−2.40 (m, 2H), 2.28−2.14 (m, 2H), 1.37 (d, J = 6.5 Hz, 6H). HRMS (ESI) m/z calcd for $[C_{34}H_{37}N_5O_2 + H]^+$, 548.3026; found, 548.3021. HPLC: 99.2% at 270 nM.

*(1R,2S)-(E)-2-(3-(4-(1-Methylpiperidin-4-yloxy)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate Salt (**57**).* The title compound was synthesized according to the general method E using **17a** (100 mg, 0.25 mmol) and (*E*)-1-methyl-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)phenoxy)-piperidine (105 mg, 0.3 mmol). Purification by reverse phase prep-HPLC gave the title compound as a yellow powder (35 mg, 25%). ^{1}H NMR (CD_3OD) δ 7.99−7.97 (m, 1H), 7.63−7.56 (m, 2H), 7.50−7.42 (m, 2H), 7.36−7.28 (m, 1H), 7.10−7.01 (m, 4H), 6.97−6.91 (m, 1H), 6.59 (t, J = 7.7 Hz, 1H), 5.99 (d, J = 7.8 Hz, 1H), 4.84−4.79 (m, 0.5H), 4.65−4.60 (m, 0.5H), 3.65−3.62 (m, 1H), 3.47−3.34 (m, 2H), 3.25−3.13 (m, 1H), 2.94 (s, 3H), 2.44−2.40 (m, 1H), 2.32−2.01 (m, 5H), 1.96−1.81 (m, 1H). LCMS (ESI) m/z calcd for $[C_{31}H_{30}N_4O_2 + H]^+$, 491.2447; found, 491.2446. Optical rotation $[\alpha]^{22}_D = -154°$ (c 0.43, MeOH). HPLC: 98.9% at 270 nM.

*(1R,2S)-2-(3-((E)-4-((1-Isopropylpiperidin-4-yl)oxy)styryl)-1H-indazol-6-yl)spiro[cyclopropane-1,3′-indolin]-2′-one 2,2,2-trifluoroacetate (**58**).* The title compound was synthesized according to the general method E using **17a** (100 mg, 0.25 mmol) and ((*E*)-1-isopropyl-4-(4-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)-phenoxy)piperidine (111 mg, 0.3 mmol). Purification by reverse phase prep-HPLC gave the title compound as a yellow powder (73 mg, 49%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.98 (d, J = 8.0 Hz, 1H), 7.55−7.64 (m, 2H), 7.42−7.50 (m, 2H), 7.27−7.36 (m, 1H), 6.98−7.09 (m, 4H), 6.94 (d, J = 7.8 Hz, 1H), 6.58 (t, J = 7.4 Hz, 1H), 5.99 (d, J = 7.3 Hz, 1H), 4.83 (bs, 1H), 3.60−3.54 (m, 2H), 3.41−3.35 (m, 2H), 3.29−3.17 (m, 1H), 2.47−2.43 (m, 1H), 2.37−2.10 (m, 5H), 1.94−1.88 (m, 1H), 1.33−1.44 (m, 6H). HRMS (ESI) m/z calcd for $[C_{33}H_{34}N_4O_2 + H]^+$, 519.2760; found, 519.2758. Optical rotation: $[\alpha]^{23}_D = -127°$ (c 0.48, MeOH). HPLC: 95.6% at 270 nM.

Biochemical Assays. Active PLK4 was purified and used to measure PLK4 activity, using an indirect ELISA detection system, as previously described.[17] PLK1, PLK2, PLK3, AURKA, and AUKB/INCENP compound inhibition were determined using FRET-based homogeneous assay kits from Invitrogen (Burlington, Ontario, Canada). Recombinant kinase enzymes were purchased from Invitrogen, with the exception of AURKB/INCENP, which was purchased from Millipore (Billerica, MA). The assays were performed according to the manufacturer's specifications with ATP concentrations of 25, 60, and 80 μM, respectively, for PLK1, PLK2, and PLK3 and ATP concentrations of 20 and 128 μM, respectively, for AURKA and AURKB/INCENP.

Compound inhibition was determined at either a fixed concentration (10 μM) or at a variable inhibitor concentration (typically 50−0.1 μM in a 10-point dose response titration). Compounds were mixed with enzyme prior to addition of ATP and the activity remaining quantified using the above-described activity assay. The % inhibition of a compound was determined using the following formula; % inhibition = 100(1 − (experimental value − background value)/(high activity control − background value)). The IC_{50} value was determined using a nonlinear four-parameter logistic curve fit (XLfit4, IDBS, Guildford, Surrey, UK) with the formula: $(A + (B/(1 + ((x/C)^D))))$, where A = background value, B = range, C = inflection point, D = curve fit parameter. Unless otherwise designated, the kinase IC_{50} values given in Tables 1, 2, and 3 are the results of a single determination. For values determined by averaging three replicates, the standard deviation is given in the PLK4 IC_{50} column.

The apparent K_is for compounds **46**, **47**, and **48** against PLK4 were estimated to be 0.10, 0.73, and 0.26 nM, respectively, by varying the ATP concentration in a series of PLK4 inhibition experiments at 0, 2.0, and 10 μM and applying nonlinear regression analysis (see Supporting Information).

The selectivity of inhibitors **46**, **47**, and **48** against 274 protein kinases was assessed by Millipore (Dundee, Scotland) using a radiometric assay. Inhibition was determined at 0.1 μM inhibitor concentration with ATP at K_m; % inhibition was determined as stated in the preceding paragraph (see Supporting Information). For compound **48**, the inhibition constants (IC_{50}s) were determined against selected kinases: TIE2, TRKA, and TRKB.

Fluorogenic cytochrome P450 inhibition studies were conducted at 37 °C in 96-well plates, as previously described,[17] for CYP 1A2, CYP 2D6, CYP 2C9, CYP 2C19, and CYP 3A4 (BFC and DBF substrates).

Cell Viability Assay (GI_{50}). MDA-MB-468, MCF-7, HCC1954, MDA-MB-231, SKBr-3, Cal-51, and BT-20 breast cancer cells were seeded into 96-well plates at 3000, 4000, 4000, 2500, 4000, 3000, and 6000 cells per 80 μL, respectively, 24 h before compound overlay and cultured at 37 °C and 5% CO_2. Compounds were prepared as 10 mM stocks in 100% DMSO. Each 10 mM stock was diluted with DMEM (Dulbecco's Modified Eagle's Medium) cell growth medium (Invitrogen, Burlington, ON, Canada) containing 10% FBS (fetal bovine serum) such that the final concentrations ranged from 50 nM to 250 μM. Aliquots (20 μL) from each concentration were overlaid to 80 μL of preseeded cells to achieve final concentrations of 10 nM to 50 μM. After 5 d, the cells were fixed in situ by gently removing the culture media and adding 50 μL of ice-cold 10% trichloroacetic acid (TCA) per well and incubation at 4 °C for 30 min. The plates were washed with water five times and allowed to air-dry for 5 min. Then 50 μL of 0.4% (w/v) sulforhodamine B (SRB) (Sigma, Oakville, ON, Canada) solution in 1% (v/v) acetic acid was added to each well, followed by incubation for 30 min at rt. The plates were washed four times with 1% acetic acid to remove unbound SRB and air-dried for 5 min. The SRB was solubilized with 100 μL of 10 mM Tris pH 10.5

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

per well, and absorbance was read at 570 nm using a SpectraPlus microplate reader (Molecular Devices Corporation). The percentage (%) of relative inhibition of cell viability was calculated by comparing to DMSO treated only cells with the maximal response pegged at 100%. GI_{50}s (concentrations required to inhibit growth by 50%) were calculated using GraphPad PRISM software (GraphPad Software, Inc., San Diego, CA, USA).

In a similar manner, lung (A549), ovarian (OVCAR-3), and colon (SW620, Colo-205, and HCT116+/+) cancer cell lines were seeded at 1500, 6000, 1500, 3000, and 800 cells, respectively, overlaid with compounds **45**, **46**, and **48**, incubated (5 d), fixed, and read using the SRB assay.

Microsomal Stability Assay. Microsomal half-life ($T_{1/2}$) for selected compounds (Table 3) was determined with human (h), dog (d), rat (r), and mouse (m) liver microsomes as previously described.[23] Curve fitting was used to determine k (first-order rate constant) using an exponential fit; the half-life was calculated as microsomal $T_{1/2} = \ln(2)/k$.

Mouse Plasma Levels. Adult female athymic CD1 nude mice (Charles River) were used in the experiments; all animal experiments were approved by UHN Animal Care Committee. Dosing solutions were prepared by dissolving the compound in 30% PEG400/70% deionized water such that the dose volume was 10 mL/kg of animal body weight. In some instances, brief sonication or vortexing and/or gentle warming was required to ensure dissolution and/or dispersion. Nine mice were dosed with compounds at 10 mg/kg (free base equivalents) via oral gavage by standard methods. Saphenous vein blood was collected from three mice per time point over an 8 h period. The plasma samples were analyzed for drug and internal standard via LCMS/MS protocol using a Waters (Millford, MA, USA) UPLC coupled to either a Q-ToF Premier, or a XevoTQ mass spectrometer. The pharmacokinetic parameters were calculated using Microsoft Excel with pharmacokinetic add-in functions from Usansky et al.[50] Parameters were calculated using plasma concentration time data for composite averages.

Xenograft Models. MDA-MB-468 breast cancer cells (Basal, HER-2 negative), A549 lung cancer cells, and SW620 colon cancer cells were purchased from ATCC (American Type Culture Collections) and cultured in DMEM (Dulbecco's Modified Eagle Medium, purchased from GIBCO) supplemented with 10% fetal calf serum. Five million MDA-MB468, 5 million A549, and 3 million SW620 were injected subcutaneously into the right flank of 6−8 week old female CB-17 SCID mice supplied by Charles River. When the tumor volume reached 80−120 mm^3, mice were randomized into groups (n = 8) and received either vehicle or compound at the doses indicated. Compounds **46**, **47**, and **48** were weighed and dissolved in 30% PEG 400/70% water. The compound was dispensed in aliquots and stored at −20 C for duration of the study, and each aliquot was thawed at rt before each dose. The mice were dosed via oral gavage daily for 21 d. Tumor volume and body weight were measured at least three times weekly. Efficacy data were graphically represented as the mean tumor volume. Tumor volume was calculated by the following formula: $x^2y/2$. Percent tumor growth inhibition after initiation of treatment with compound was calculated by TGI = 100 × 1 − (tumor $volume_{final}$ − tumor $volume_{initial}$ for compound treated group)/(tumor $volume_{final}$ − tumor $volume_{initial}$ for compound control group).

HCT116 colon cancer cells were purchased, cultured, and injected (1 million HCT116 cancer cells) into the right flank of 6−8 week old female athymic CD1 nude mice (Charles River) as described above. Upon reaching a tumor volume of 80−120 mm^3, mice were randomized into groups (n = 8) and received either vehicle or weighed compounds **46**, **47**, and **48** dissolved in 30% PEG 400/70% water as described above. The mice were dosed via oral gavage on days 1, 5, 9, 13, 17, and 21 and sacrificed on day 35. Tumor volume, body weight, and percent growth inhibition were determined as described above.

Rat and Dog Pharmacokinetic Studies. The pharmacokinetic studies were performed by Shanghai Chempartner, 720 Cailun Road, Pudong New Area, Shanghai, China. Compounds were dissolved in 10% NMP/40% PEG/50% water. Male SD rats (200−230 g, purchased from SLAC Laboratory Animal Co. LTD) were administered IV (foot dorsal vein) and PO (oral gavage); blood samples were collected via cannulated jugular veins and stored frozen until analysis.

Seven male beagle dogs (6.5−9 kg), were purchased from Suzhou Xishan Zhongke laboratory animal breeding center and with Qualification no. SCXK 2007-0005, 0014369. The IV dose (3 dogs) was conducted via cephalic vein injection, PO (3 dosage), via intragastric administration. Approximately 0.5 mL blood/time point was collected from cephalic or saphenous veins into precold EDTA tubes. Blood samples were put on wet ice and centrifuged at 4 °C to obtain plasma within 30 min of sample collection and stored frozen until analysis. One animal, not placed on study, was used for collection of blank blood (5−10 mL). The resulting blank plasma was used for the development of the bioanalytical method and standard curve.

Diluted time point samples were analyzed by LCMSMS-02 (API 4000, triple quadrupole) using a standard curve. The PK parameters were determined by a noncompartmental model using WinNonlin Professional 5.2.

■ ASSOCIATED CONTENT

Supporting Information

Double reciprocal plot of PLK4 inhibition by compound **48**; synthesis of compound intermediates; atomic coordinates, bond lengths and angles, and anisotropic displacement parameters for an X-ray structure of compound **33**; inhibition data for the shortlisted compounds (**46**, **47**, and **48**) at 0.1 μM against a 274 member kinase panel. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 416-581-7620. E-mail: hpauls@uhnresearch.ca.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. Homer Pearce for helpful discussion during the course of the work and Dr. Alan Lough, X-ray Crystallography, University of Toronto, for X-ray data collection and structure refinement. The Princess Margaret Cancer Foundation, the Canadian Institute of Health Research, and the Canadian Foundation for Innovation are acknowledged for financial support.

■ ABBREVIATIONS USED

aq, aqueous; anhyd, anhydrous; Ar, argon; ATP, adenosine-5′-triphosphate; Boc, *tert*-butoxycarbonyl; b, broad; calcd, calculated; CYP, cytochrome P450; d, doublet; DBU, 1,8-diazabicycloundec-7-ene; DCM, dichloromethane; DIPEA, diisopropylethylamine; DMAP, 4-dimethyl-aminopyridine; DME, dimethoxyethane; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; dppf, 1,1′-bis(diphenylphosphino) ferrocene; FLT3, FMS-related tyrosine kinase 3; GI_{50}, half-maximal cell growth inhibitory concentration; GST, glutathione *S*-transferase; h, hour; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HOBt, hydroxybenzotriazole; HMBC, heteronuclear multiple-bond correlation spectroscopy; HPLC, high performance liquid chromatography; HRMS, high-resolution mass spectrometry; IP, intraperitoneal; KDR, kinase insert domain receptor; LCMS, liquid chromatography coupled to mass spectrometry; MBP, myelin basic protein; min, minute; m, multiplet; MS ESI, electrospray ionization mass spectrometry; NMR, nuclear magnetic resonance; NBS, *N*-bromosuccinimide; PAMPA, parallel artificial membrane permeability assay; PBS, phosphate buffered saline; PCR polymerase chain

dx.doi.org/10.1021/jm5005336 | J. Med. Chem. XXXX, XXX, XXX−XXX

reaction; PDB, Protein Data Bank; PEG400, poly(ethylene glycol) 400; pin, pinacol; PMB, *p*-methoxybenzyl; PPB, plasma protein binding; prep, preparative; QSAR, quantitative structure−activity relationship; RBF, round-bottomed flask; RNAi, RNA interference; rt, room temperature; RP, reverse phase; s, singlet; satd, saturated; SEM, 2-(trimethylsilyl)ethoxy]-methyl; siRNA, small interfering RNA; SMs, starting materials; t, triplet; TBAB, tetra-*n*-butylammonium bromide; TEA, triethylamine; temp, temperature; TFA, trifluoroacetic acid; TGI, tumor growth inhibition; THF, tetrahydrofuran; TLC, thin layer chromatography; TMT, 2,4,6-trimercaptotriazine; q, quartet; UPLC, ultra performance liquid chromatography; UHN IACUC, University Health Network Institutional Animal Care and Use Committee

REFERENCES

(1) Barr, F. A.; Silljé, H. H. W.; Nigg, E. A. Polo-like kinases and the orchestration of cell division. *Nature Rev. Mod. Cell. Biol.* **2004**, *5*, 429−440.

(2) Archambault, V.; Glover, D. M. Polo-like kinases: conservation and divergence in their functions and regulation. *Nature Rev. Mol. Cell Biol.* **2009**, *10*, 265−275.

(3) Schoffski, P. Polo-like kinase inhibitors in preclinical and early clinical development. *Oncologist* **2009**, *14*, 559−570.

(4) Sillibourne, J. E.; Bornens, M. Polo-like kinase 4: the odd one out of the family. *Cell Div.* **2010**, *5*, 25−34.

(5) Bettencourt-Dias, M.; Rodrigues-Martins, A.; Carpenter, L.; Riparbelli, M.; Lehmenn, L.; Gatt, M. K.; Carmo, N.; Balloux, F.; Callani, G.; Glover, D. M. SAK/PLK4 is required for centriole duplication and flagella development. *Curr. Biol.* **2005**, *15*, 2199−2207.

(6) Habedanck, R.; Stierhof, Y.-D.; Wilkinson, C. J.; Nigg, E. A. The Polo kinase PLK4 functions in centriole duplication. *Nature. Cell Biol.* **2005**, *7*, 1140−1146.

(7) Kleylein-Sohn, J.; Westendorf, J.; Le Clech, M.; Habedanck, R.; Stierhof, Y.-D.; Nigg, E. A. Plk4-induced centriole biogenesis in human cells. *Dev. Cell* **2007**, *13*, 190−202.

(8) Ko, M. A.; Rosario, C. O.; Hudson, J. W.; Kulkarni, S.; Pollett, A.; Dennis, J. W.; Swallow, C. J. Plk4 haploinsufficiency causes mitotic infidelity and carcinogenesis. *Nature Genet.* **2005**, *37*, 883−888.

(9) Basto, R.; Brunk, K.; Vinadogrova, T.; Peel, N.; Franz, A.; Khodjakov, A.; Raff, J. W. Centrosome amplification can initiate tumorigenesis in flies. *Cell* **2008**, *133*, 1032−1042.

(10) Liu, L.; Zhang, C. Z.; Cai, M.; Fu, J.; Chen, G. G.; Yun, J. Downregulation of polo-like kinase 4 in hepatocellular carcinoma associates with poor prognosis. *PLoS One* **2012**, *7*, e41293.

(11) Hu, Z.; Fan, C.; Oh, D. S.; Marron, J. S.; He, X.; Qaqish, B. F.; Livasy, C.; Carey, L. A.; Reynolds, E.; Dressler, L.; Nobel, A.; Parker, J.; Ewend, M. G.; Sawyer, L. R.; Wu, J.; Liu, Y.; Nanda, R.; Tretiakova, M.; Orrico, A. R.; Dreher, D.; Palazzo, J. P.; Perreard, L.; Nelson, E.; Mone, M.; Hansen, H.; Mullins, M.; Quackenbush, J. F.; Ellis, M. J.; Olopade, O. I.; Bernard, P. S.; Perou, C. M. The molecular portraits of breast tumors are conserved across microarray platforms. *BMC Genomics* **2006**, *7*, 96−108.

(12) van di Vijver, M. J.; He, Y. D.; van 't Veer, L.; Dai, H.; Hart, A. A. M.; Voskuil, D. W.; Schreiber, G. J.; Peterse, J. L.; Roberts, C.; Marton, M. J.; Parrish, M.; Atsma, D.; Witteveen, A.; Glas, A.; Delahaye, L.; van der Velde, T.; Bartelink, H.; Rodenhuis, S.; Rutgers, E. T.; Friend, S. H.; Bernards, R. A gene-expression signature as a predictor of survival in breast cancer. *N. Engl. J. Med.* **2002**, *347*, 1999−2009.

(13) Miller, L. D.; Smeds, J.; George, J.; Vega, V. B.; Vergara, L.; Ploner, A.; Pawitan, Y.; Hall, P.; Klaar, S.; Liu, E. T.; Bergh, J. An expression signature for p53 status in human breast cancer predicts mutation status, transcriptional effects, and patient survival. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102*, 13550−13555.

(14) Mason, J.; Wei, S.; Luo, S.; Nadeem, V.; Kiarash, R.; Huang, P.; Awrey, A.; Leung, G.; Beletskaya, I.; Feher, M.; Forrest, B.; Laufer, R.; Sampson, P.; Li, S.-W.; Liu, Y.; Lang, Y.; Pauls, H. W.; Mak, T. W.; Pan, J. G. Inhibition of Polo-like kinase 4 as an anti-cancer strategy. AACR 102nd Annual Meeting, Orlando, FL, April 2−6, 2011, LB-215.

(15) Mak, T. W. Targeting the Cell Cycle in Cancer: TTK (MPS1) and PLK4 as Novel Mitotic Targets. AACR 103rd Annual Meeting, Chicago, IL, April 1−5, 2012.

(16) Pauls, H. W. PLK4 inhibitors of novel structure as potent anti-proliferative agents. 241st ACS National Meeting, Anaheim, CA, March 27−31, 2011, MEDI 189.

(17) Laufer, R.; Forrest, B.; Li, S.-W.; Sampson, P.; Liu, Y.; Edwards, L.; Lang, Y.; Awrey, D.; Mao, V.; Plotnikova, O.; Leung, G.; Hodgson, R.; Beletskaya, I.; Mason, J.; Wei, S.; Luo, X.; Nadeem, V.; Feher, M.; Kiarash, R.; Green, E.; Mak, T. W.; Pan, J. G.; Pauls, H. W. The discovery of PLK4 inhibitors: (*E*)-3-((1*H*-indazol-6-yl)methylene)-indolin-2-ones as novel anti-proliferative agents. *J. Med. Chem.* **2013**, *56*, 6069−6087.

(18) Karaman, M. W.; Herrgard, S.; Treiber, D. K.; Gallant, P.; Atteridge, C. E.; Campbell, B. T.; Chan, K. W.; Ciceri, P.; Davis, M. I.; Edeen, P. T.; Faraoni, R.; Floyd, M.; Hunt, J. P.; Lockhart, D. J.; Milanov, Z. V.; Morrison, M. J.; Pallares, G.; Patel, H. K.; Pritchard, S.; Wodicka, L. M.; Zarrinkar, P. P. A quantitative analysis of kinase inhibitor selectivity. *Nature Biotechnol.* **2008**, *26*, 127−132.

(19) Sebolt-Leopold, J. S.; English, J. M. Mechanisms of drug inhibition of signalling molecules. *Nature* **2006**, *441*, 457−462.

(20) Bain, J.; McLauchlan, H.; Elliott, M.; Cohen, P. The specificities of protein kinase inhibitors: an update. *Biochem. J.* **2003**, *371*, 199−204.

(21) Knight, Z. A.; Shokat, K. M. Features of selective kinase inhibitors. *Chem. Biol.* **2005**, *12*, 621−637.

(22) McInnes, C.; Mezna, M.; Fischer, P. M. Progress in the discovery of polo-like kinase inhibitors. *Curr. Top. Med. Chem.* **2005**, *5*, 181−197.

(23) Reindl, W.; Yuan, J.; Kramer, A.; Strebhardt, K.; Berg, T. Inhibition of polo-like kinase 1 by blocking polo-box domain-dependent protein−protein interactions. *Chem. Biol.* **2008**, *15*, 459−466.

(24) Sampson, P. B.; Liu, Y.; Patel, N. K.; Feher, M.; Forrest, B.; Li, S.-W.; Edwards, L.; Laufer, R.; Lang, Y.; Ban, F.; Awrey, D.; Mao, G.; Plotnikova, O.; Leung, G.; Hodgson, R.; Mason, J.; Wei, X.; Kiarash, R.; Green, E.; Qiu, W.; Chirgadze, N. Y.; Mak, T. W.; Pan, G.; Pauls, H. W. The discovery of PLK4 inhibitors: design and optimization of spiro[cyclopropane-1,3′[3*H*]indol]-2′(1′*H*)-ones as anti-cancer agents. *J. Med. Chem.* DOI 10.1021/jm500537u.

(25) Li, A.-H.; Dai, L.-X.; Aggarwal, V. K. Asymmetric ylide reactions: epoxidation, cyclo-propanation, aziridination, olefination, and rearrangement. *Chem. Rev.* **1997**, *97*, 2341−2372.

(26) Aggarwal, V. K.; Alonso, E.; Fang, G.; Ferrara, M.; Hynd, G.; Porcelloni, M. Application of chiral sulfides to catalytic asymmetric aziridination and cyclopropanation with in situ generation of the diazo compound. *Angew. Chem., Int. Ed.* **2001**, *40*, 1433−1436.

(27) Marti, C.; Carreira, E. M. Total synthesis of (−)-spirotryprostatin B: synthesis and related studies. *J. Am. Chem. Soc.* **2005**, *127*, 11505−11515.

(28) Yun, G.; Sharpless, K. B. Vicinal diol cyclic sulfates. Like epoxides only more reactive. *J. Am. Chem. Soc.* **1988**, *110*, 7538−7539.

(29) Pauls, H. W. The discovery of PLK4 inhibitors as novel anti-proliferative agents. Canadian Chemistry Conference & Exhibition, Quebec City, QC, May 26−29, 2013, BM7-00029.

(30) Haddach, A. A.; Kelleman, A.; Deaton-Rewolinski, M. V. An efficient method for the *N*-debenzylation of aromatic heterocycles. *Tetrahedron Lett.* **2002**, *43*, 399−402.

(31) Williams, R. M.; Kwast, E. Carbanion-mediated oxidative deprotection of non-enolizable benzylated amides. *Tetrahedron Lett.* **1989**, *30*, 451−454.

(32) Performed by Lotus Separations, Princeton NJ.

(33) Kolb, H. C.; VanNiewenhze, M. S.; Sharpless, K. B. Catalytic assymmetric dihydroxylation. *Chem. Rev.* **1994**, *94*, 2483−2547.

(34) Hunter, C. A.; Sanders, J. K. M. The nature of $\pi-\pi$ interactions. *J. Am. Chem. Soc.* **1990**, *112*, 5525−5534.

(35) Bhosale, S. S.; Joshi, P. L.; Rae, A. S. A convenient procedure for the preparation of 2-bromo-1-phenylethanol. *Org. Prep. Proced. Int.* **2009**, *24*, 695−696.
(36) Weinstock, J.; Ladd, D. L.; Wilson, J. W.; Brush, C. K.; Yim, N.C. F.; Gallagher, G., Jr.; McCarthy, M. E.; Silvestri, J.; Sarau, H. M.; Flaim, K. E.; Ackerman, D. M.; Setler, P. E.; Tobia, A. J.; Hahns, R. A. Synthesis and renal vasodilator activity of some dopamine agonist l-aryl-2,3,4,5-tetrahydro-1*H*-3-benzazepine-7,8-diols: halogen and methyl analogues of fenoldopam. *J. Med. Chem.* **1986**, *29*, 2315−2325.
(37) Smolinsky, G. Formation of azacyclopropenes by pyrolysis of vinyl azides. *J. Org. Chem.* **1962**, *27*, 3557−3559.
(38) Manfredi, M. G.; Ecsedy, J. A.; Meetze, K. A.; Balani, S. K.; Burenkova, O.; Chen, W.; Galvin, K. M.; Hoar, K. M.; Huck, J. J.; LeRoy, P. J.; Ray, E. T.; Sells, T. B.; Stringer, B.; Stroud, S. G.; Vos, T. J.; Weatherhead, G. S.; Wysong, D. R.; Zhang, M.; Bolen, J. B.; Claiborne, C. F. Antitumor activity of MLN8054, an orally active small-molecule inhibitor of Aurora A kinase. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 4106−4111.
(39) Innocenti, P.; Cheung, K-M.J.; Solanki, S.; Mas-Droux, C.; Rowan, F.; Yeoh, S.; Boxall, K.; Westlake, M.; Pickard, L.; Hardy, T.; Baxter, J. E.; Aherne, G. W.; Bayliss, R.; Fry, A. M.; Hoelder, S. Design of potent and selective hybrid inhibitors of the mitotic kinase Nek2: SAR, structural biology and cellular activity. *J. Med. Chem.* **2012**, *55*, 3228−3241.
(40) Mason, J. M.; Lin, D.C-C.; Wei, X.; Che, Y.; Yao, Y.; Kiarash, R.; Fletcher, G. C.; Bray, M. R.; Pan, G. J.; Mak, T. W. Inhibition of PLK4 as a therapeutic strategy for genomically unstable cancers deficient for PTEN. AACR-NCI-EORTC International Conference on Molecular Targets & Cancer Therapeutics, Boston, MA, October 19−23, 2013, B267.
(41) Mason, J. M.; Lin, D.C-C.; Wei, X.; Che, Y.; Yao, Y.; Kiarash, R.; Cescon, D. W.; Fletcher, G. C.; Awrey, D. E.; Bray, M. R.; Pan, G.; Mak, T. W. Functional characterization of CFI-400945, an inhibitor of PLK4, as a potential anticancer agent. *Cancer Cell* **2014**, in press.
(42) *ACD/Structure Designer*, version 12.01; Advanced Chemistry Development, Inc.: Toronto, ON, Canada, 2012.
(43) Nakagawara, A.; Azar, C. G.; Scavarda, N. J.; Brodeur, G. M. Expression and function of TRK-B and BDNF in human neuroblastomas. *Mol. Cell. Biol.* **1994**, *14*, 759−767.
(44) Peters, K. G.; Coogan, A.; Berry, D.; Marks, J.; Iglehart, J. D.; Kontos, C. D.; Rao, P.; Sankar, S.; Trogan, E. Expression of Tie2/Tek in breast tumor vasculature provides a new marker for evaluation of tumour angiogenesis. *Br. J. Cancer* **1998**, *77*, 51−56.
(45) Fister, R.; Panetta, J. C. Optimal control applied to cell-cycle-specific cancer chemotherapy. *SIAM J. Appl. Math.* **2000**, *60*, 1059−1072.
(46) Venugopal, B.; Baird, R.; Kristeleit, R. S.; Plummer, R.; Cowan, R.; Stewart, A.; Fourneau, N.; Hellemans, P.; Elsayed, Y.; Mcclue, S.; Smit, J. W.; Forslund, A.; Phelps, C.; Camm, J.; Evans, T. R. J.; de Bono, J. S.; Banerji, U. A phase I study of quisinostat (JNJ-26481585), an oral hydroxamate histone deacetylase inhibitor with evidence of target modulation and antitumor activity, in patients with advanced solid tumors. *Clin. Cancer Res.* **2013**, *19*, 4262−4272.
(47) Wei, P.; Walls, M.; Qiu, M.; Ding, R.; Denlinger, R. H.; Wong, A.; Tsaparikos, K.; Jani, J. P.; Hosea, N.; Sands, M.; Randolph, S.; Smeal, T. Evaluation of selective γ-secretase inhibitor PF-03084014 for its antitumor efficacy and gastrointestinal safety to guide optimal clinical trial design. *Mol. Cancer Ther.* **2010**, *9*, 1618−1628.
(48) Awar, O.; Duvic, M. Treatment of transformed mycosis fungoides with intermittent low-dose Gemcitabine. *Oncology* **2007**, *73*, 130−135.
(49) *MOE (Molecular Operating Environment)*, version 2005.06; Chemical Computing Group Inc.: 1010 Sherbrooke St. West, Suite 910, Montreal, Quebec, H3A 2R7, Canada, 2005.
(50) Usansky, J. I.; Desai, A.; Tang-Liu, D. PK Functions for Microsoft Excel. *Pharmacokinetic Software*; David W. A. Bourne, 2001−2003; http://www.boomer.org/pkin/soft.html, Accessed November, 2008.