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Discovery and synthesis of novel 4-aminopyrrolopyrimidine Tie-2 kinase inhibitors for the treatment of solid tumors

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ABSTRACT

The synthesis and biological evaluation of novel Tie-2 kinase inhibitors are presented. Based on the pyrrolopyrimidine chemotype, several new series are described, including the benzimidazole series by linking a benzimidazole to the C5-position of the 4-amino-pyrrolopyrimidine core and the ketophenyl series synthesized by incorporating a ketophenyl group to the C5-position. Medicinal chemistry efforts led to potent Tie-2 inhibitors. Compound **15**, a ketophenyl pyrrolopyrimidine urea analog with improved physicochemical properties, demonstrated favorable in vitro attributes as well as dose responsive and robust oral tumor growth inhibition in animal models.

Solid tumor growth and invasion depend on an adequate blood supply, and angiogenesis remains an area of high importance for anticancer drug discovery.[1] Tie-2 is a receptor tyrosine kinase (RTK) that is essential for the formation of the embryonic vasculature and is strongly implicated in tumor angiogenesis.[2] Whereas VEGF and other RTKs deliver the primary proliferation signals to the endothelium, Tie-2 signals largely regulate vessel integrity, and play roles in the dynamic modeling of vessels required for angiogenesis.[3] Tie-2 and its various angiopoeitin ligands (Ang-1, Ang-2 and Ang-4) can either promote vessel integrity and decrease permeability (via Ang-1 stimulation of Tie-2),[3] or can drive vessel destabilization and permeabilization as a prelude to sprouting and angiogenesis (Ang-2 inhibition of Tie-2).[4] It has been suggested that alteration of Tie-2 activity in either direction: constitutive activation leading to an overly stable vasculature, or inhibition leading to protracted destabilization, could result in impediments to both tumor angiogenesis and tumor growth.[5]

Our objective was to develop potent novel Tie-2 inhibitors with good selectivity against other kinases closely involved in angiogenesis. The desired inhibitors would need to have adequate ADMET attributes for safe, oral cancer treatment. Prior to our effort, pyrrolopyrimidines had been reported to bind to the Tie-2 kinase domain. However, specific details were not available with regard to Tie-2 kinase/cellular potency, selectivity, SAR, ADMET and in vivo attributes. For example, compound **1** (Fig. 1), with a chlorine substituted phenyl linked directly to the 5-position of the 4-amino-7-cyclopentyl-pyrrolopyrimidine core and an aryl sulfonamide attached to the

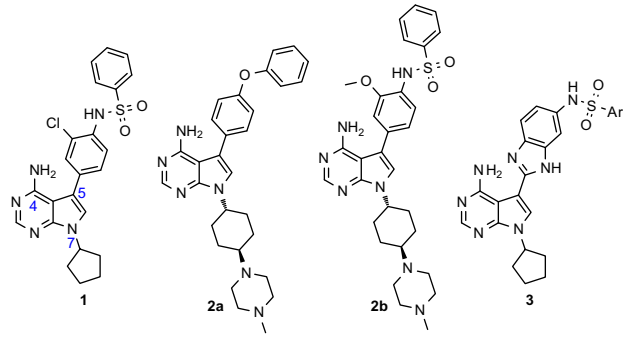

Figure 1. Tie-2 active pyrrolopyrimidines.

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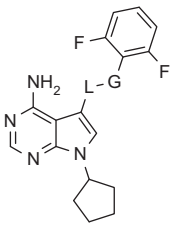
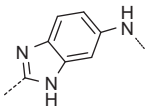
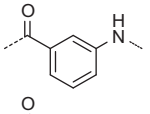
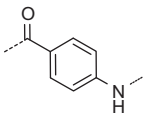
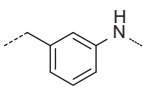
para-position of the phenyl linker, was reported to bind to the catalytic domain of human Tie-2 in a co-crystal structure,[6] no further information was available in the literature. Pyrrolopyrimidines have also been reported to inhibit Lck[7] and Src[8] with weak Tie-2 activity. For example, compound **2a** had an IC_{50} of 15 nM against Lck and 250 nM against Tie-2 at 1 mM ATP.[7a] Furthermore, compound **2b** was reported to inhibit Tie-2 with an IC_{50} of 137 nM, whereas its IC_{50}s against Lck, Src and kdr were greater than 3000 nM.[7b] In recent years there have been several reports on non-pyrrolopyrimidine based Tie-2 inhibitors,[9] but few with the kinase selectivity attributes of our interest at the time. Herein we report the discovery and synthesis of novel pyrrolopyrimidines as potent Tie-2 inhibitors with good selectivity against other key angiogenesis kinases, adequate ADMET properties, and robust tumor growth inhibition in xenograft models.

We designed and synthesized a number of analogs with new linkers and eventually identified a few active pyrrolopyrimidine analogs with a benzimidazole linker and arylsulfonamide head groups (**3**, Table 1). For example, compound **4**, with a 2,6-difluoro-phenylsulfonamide head group, had Tie-2 kinase and cellular IC_{50}s of 568 and 518 nM, respectively.[10] The corresponding urea analog compound **5** was then prepared which proved to be more potent in the Tie-2 kinase and cellular assays, with IC_{50}s of 241 and 112 nM, respectively. Subsequently, we designed and synthesized the ketophenyl linker and synthesized the corresponding *meta*-sulfonamide **6** and *meta*-urea **7**. Similar to the benzimidazole analogs, the urea analog **7** was more potent than the corresponding sulfonamide analog **6**. This trend was more pronounced in the cellular assay, with an IC_{50} of 67 nM for compound **7** and about fourfold reduced activity (253 nM) of the corresponding sulfonamide analog **6**. Alternative substitution positions on both ketophenyl and benzimidazole linkers resulted in analogs of weaker activity. For example, the *para*-substituted ketophenyl analogs **8** and **9** were less active than the corresponding *meta*-analogs **6** and **7**, respectively. Furthermore, extending the head functional group into carbamates, carboxamides and amines (G = CO(O), CO, CH_2) also led to diminished potency in general. One of the hypotheses employed in the design at this stage was to minimize the rotational freedom of the bond connecting the linker to the pyrrolopyrimidine core. For example, a benzimidazole linker may bring in potential rigidity via an intramolecular hydrogen-bond between a pyrimidine NH and imidazole ring N; as could a pyrimidine NH with the carbonyl O in the ketophenyl analogs. Compound **10**, where the carbonyl group is reduced to the methylene, was significantly less active (>10,000 nM). This appeared to suggest a critical role of the H-bond acceptor in this region of the molecule and was consistent with the initial hypothesis.

Table 1
Impact of linker on kinase and cellular potency



L	G	Compound	IC_{50} (nM) kinase/cell
	SO_2	**4**	568/518
	CONH	**5**	241/122
	SO_2	**6**	423/253
	CONH	**7**	361/67
	SO_2	**8**	745/769
	CONH	**9**	345/254
	SO_2	**10**	>10,000/ND

These early analogs appeared to have some selectivity against the kinases we intended to avoid the most. For example, compound **7** had kinase IC_{50} values of 3100 and >5000 nM against KDR and PDGFR, respectively. Some of them exhibited significant inhibition against Trk, a family of receptor tyrosine kinases originally identified as modulators of the maintenance and survival of neuronal cells.[11] In the last two decades, evidence has suggested that Trk kinases (TrkA and TrkB) play key roles in malignant transformation, chemotaxis, metastasis, and survival signaling in human tumors. As a result, Trk kinases have attracted significant attention as drug discovery targets for cancer and pain therapy.[12] For example, TrkB has been found to be over expressed in some small cell lung cancer and prostate cancers. Thus a dual Tie-2/Trk inhibitor might be hypothesized to provide additional efficacy against these tumors.

Compounds **4** through **7** had MW around 500, *c*log*P* of 4–5, and TPSA of 115–140 (Table 2), all approaching the upper limits for orally available drugs.[13] These molecules appeared to have reasonable permeability, but could be potential substrates for P-gp mediated efflux. For example, compound **7** had a value of 7.7×10^{-6} cm/s for the apical to basal measurement in the Caco-2 cell line, with a high Caco-2 BA/AB ratio of 3.5. In addition, rat liver microsomal stability of these compounds was poor, with unfavorably high rat liver microsomal extraction ratio (RLM Er >0.60). Furthermore, solubility of these analogs was typically very low (<5 μg/mL).

Enzyme kinetics studies carried out on some of these analogs suggested that they were reversible and ATP competitive Tie-2 kinase inhibitors. The ketoureas appeared to have a much slower off-rate than the ketosulfonamide, which could potentially translate to advantages in pharmacological effects.[14] Our initial lead optimization efforts mostly focused on improving Tie-2 potency (thereby selectivity), addressing metabolic stability, P-gp mediated efflux and solubility. Our medicinal chemistry strategy was to fully explore the SAR of head aryl group for optimal lipophilic binding interaction in this region, as well as to reduce size and lipophilicity via significant modification of the cyclopentyl tail group.

The team subsequently synthesized diverse analogs of the benzimidazole and the *meta*-ketophenyl series including sulfonamides, ureas, and a smaller number of other analogs (carbamates, carboxamides, and amines). All analogs synthesized at this stage maintained the cyclopentyl tail group for SAR continuity. Out of this effort a total of 350 analogs were made, and their potency distribution versus chemotype is plotted in Figure 2. The most potent analogs were ketophenyl ureas.

Table 2
Molecular properties of initial leads

Compound	MW	*c*log*P*	TPSA	RLM Er	Caco-2 BA/AB
4	509	4.69	140	0.69	ND
5	488	4.48	127	0.90	3.2
6	497	4.11	128	ND	4.7
7	476	4.05	115	0.83	3.5

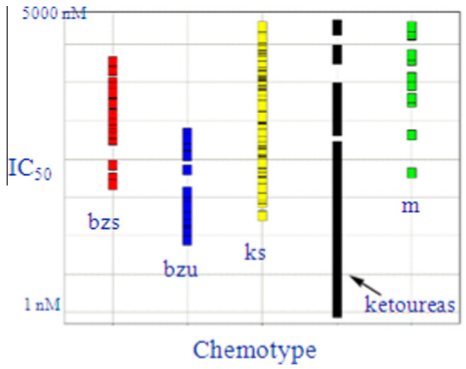


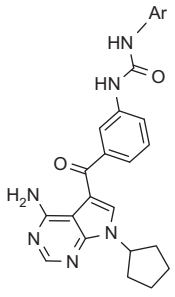
Figure 2. Chemotype versus Tie-2 IC_{50}. bzs, benzimidazole sulfonamides; bzu, benzimidazole ureas; ks, *meta*-ketophenyl sulfonamides; m, amides, carbamates, amines.

Four of the more potent cyclopentyl ketophenyl ureas, compounds **11**, **12**, **13**, and **14**, are listed in Table 3. All had lipophilic substituents (Cl or Me) at the *meta*- or *para*-position of the terminal phenyl group. The cellular IC_{50}s of these were in the low single digit nanomolar to picomolar range (4.6, 1.2, 0.89, and 0.69 nM, respectively).[15] The most potent compounds in the cell assays exhibited a hydrogen bond acceptor at the 2-position of the terminal phenyl ring (F in compound **13**, O in compound **14**), which might hydrogen bond to the adjacent urea NH thus leading to potentially favorable conformation and/or permeability. As the MW increased and *c*log*P* remained high for these analogs, RLM Er and Caco-2 BA/AB ratio were still problematically high.[16]

Medicinal chemistry efforts were then focused on the cyclopentyl tail group of the ketophenyl ureas. Correlation between RLM Er and *c*log*P* of these analogs was poor in general suggesting that multiple metabolic pathways were potentially involved. One objective was to modify the cyclopentyl group to reduce overall lipophilicity and MW of these analogs. A series of new tail groups of various lipophilicities and sizes were designed and the penultimate amine intermediates were synthesized with these tail groups installed. Each of these templates were utilized to synthesize a set of 20 ureas and 20 sulfonamides. The selection of isocyanate and sulfonyl chloride building blocks for this effort was directed by SAR results of the cyclopentyl ketophenyl series. The average RLM Er and cellular IC_{50}s of these subsets are listed in Table 4. Several observations were made from these data: (1) smaller groups (R = H, Me, *i*-Pr) were preferred over the larger groups (THP, cyclopentyl, methoxyethyl) for better microsomal stability in general; (2) the methoxyethyl tail was the least stable, in spite of a favorable *c*log*P* and moderate size, probably due to metabolic demethylation; (3) larger groups (cyclopentyl, THP, *i*-Pr) were favored in terms of Tie-2 potency. Overall, *i*-Pr analogs imparted moderately improved microsomal stability (RLM Er 0.56) while maintaining potency equivalent to cyclopenyl group (0.29 μM).

From these efforts ketophenyl urea compound **15** emerged and the attributes of **15** are listed in Figure 3. Compound **15** had low single digit nM Tie-2 cellular IC_{50} and equally potent TrkA cellular activity (TrkA kinase/cell IC_{50} of 0.4 and 4 nM, respectively). Its MW of 448, *c*log*P* of 4.7, and TPSA of 115 were all within the boundaries of oral drugs. These appeared to have a positive impact on the microsomal stability, with both rat and human liver microsomal extraction ratios close to 0.5. Compound **15** also had improved solubility (35 μg/mL) and permeability (Caco-2 BA: 9.5×10^{-6} cm/s). With a Caco-2 BA/AB ratio of 1.5, this compound was less likely a P-gp mediated efflux substrate. Inhibition of cytochrome P450 isozymes by compound **15** was low (IC_{50} >3 μM). Compound **15** had modest kinase selectivity[15] against the key oncology targets we intended to avoid. For example, its IC_{50}s against KDR, FGFR, and EGFR were >10,000 nM and PDGFR at 1400 nM. Compound **15** was negative in the Biolums AMES assays, but positive in the in vitro micronucleus assay. It also inhibited the hERG potassium ion channel with an iKr IC_{20} of 1000 nM.

Table 3
Cyclopentyl *meta*-ketophenyl ureas



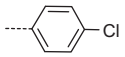
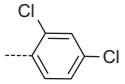
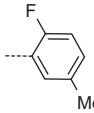
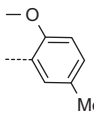
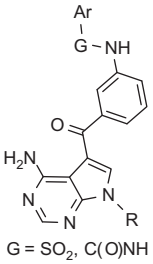
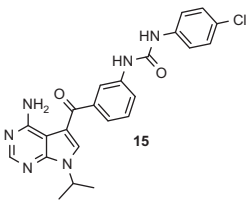
Ar	Compound	IC_{50} kinase/cell	MW	*c*log*P*	RLM Er	Caco-2 BA/AB
	**11**	45/4.6	475	5.3	0.7	17
	**12**	ND/1.2	509	5.5	ND	1.9
	**13**	12/0.89	472	4.8	0.8	9.2
	**14**	8.8/0.69	484	5	0.69	9.4

Table 4
Tail groups, average RLM Er and cellular potency



G = SO_2, C(O)NH

R	IC_{50} (uM)	RLM Er
H	0.99	0.44
Me	1.35	0.49
	0.29	0.56
O	0.24	0.65
	0.29	0.75
OMe	1.31	0.78



kinase/cell	40 / 3.7 nM
MW / cLogP / TPSA	449 / 4.7 / 115
RLM / HLM Er	0.52 / 0.46
Caco-2 BA & BA/AB	$9.5x10^{-6}$ cm/s & 1.5
Cypsinhibition IC50	>3 μM
solubility	35 μg / mL
KDR, FGFR, EGFR	>10,000 nM
PDGFR	1400 nM
Biolums AMES	negative
IV micronucleus	positive
iKr IC_{20}	1000 nM

Figure 3. Attributes of compound **15**.

Compound **15** inhibited Tie-2 phosphorylation in an ex vivo model using a cloned C6 rat glioblastoma cell line over expressing human Tie-2 via a retrovirus construct.[17] This inhibition was dose responsive and plasma concentration dependent (Fig. 4). In PK studies, 0.164 mg/kg IV dose in fed male Sprague–Dawley rats led to the observation of a clearance (Cl) of 38 mL/min/kg (54% Q), a steady state volume of distribution (VDss) of 0.9 L/kg and a $T_{1/2}$ of 0.4 h. Oral suspension dose in ultrafasted rats at 4.2 mg/kg led to a systemic C_{max} of 1715 ng/mL, T_{max} of 0.75 h, hepatic extraction (Eh) of 27 mL/min/kg (38%) and oral bioavailability (*F*%) of 100%.

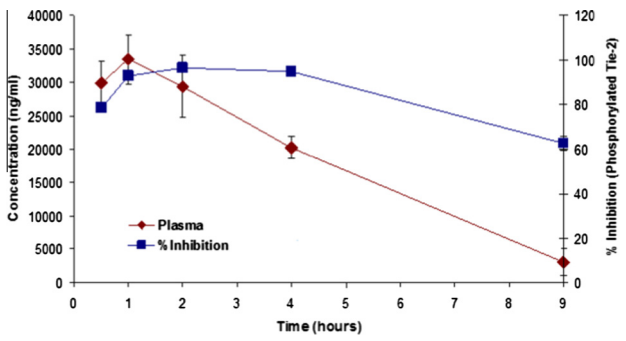


Figure 4. Inhibition of Tie-2 phosphorylation versus plasma concentration.

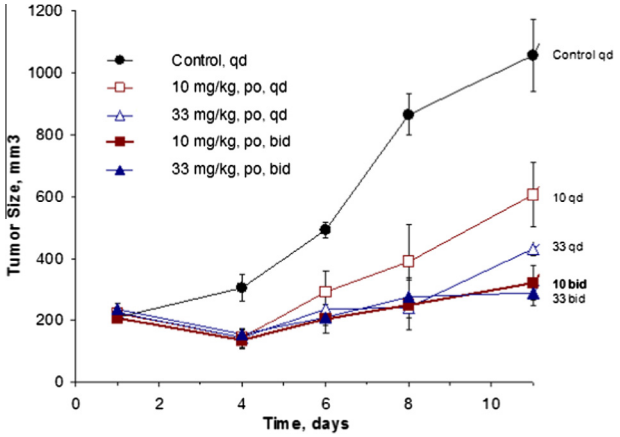
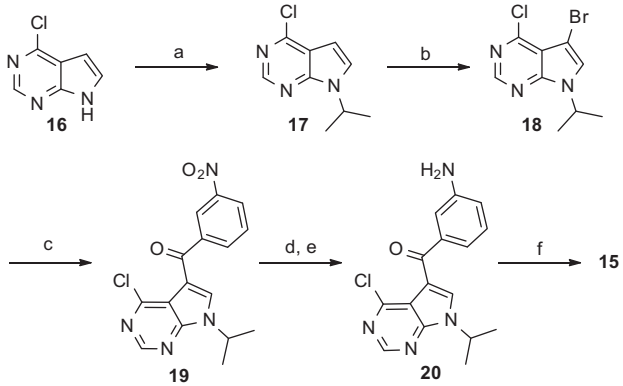


Figure 5. C6 tumor growth inhibition of compound **15**.

In tumor growth inhibition (TGI) studies, compound **15** exhibited robust efficacy in several xenograft models. For example, in the C6 (uninfected parental C6 cells lacking human Tie-2) rat glioblastoma model, compound **15** was dosed orally once daily (po qd) at 10 or 33 mg/kg through the 10 day study (Fig. 5). At 10 mg/kg po qd, ~40% TGI was achieved whereas 33 mg/kg po qd resulted in ~60% TGI. For the bid dose, 10 mg/kg furnished ~70% TGI and 33 mg/kg resulted in a slightly higher TGI. The observation that bid dosing promotes minimal enhancement of tumor inhibition at the 33 mg/kg dose relative to 10 mg/kg dose is consistent with our observations that Tie-2 inhibitors may block new blood vessel formation and tumor growth, but do not generally induce tumor shrinkage when administered as single agents. Thus, the tumor inhibitions observed for the bid doses may reflect near-maximal activity in this model. No significant loss of body weight was observed in any animal during these studies.

The synthesis of compound **20**, the penultimate intermediate to compound **15**, began with alkylation of compound **16** (Scheme 1).[18] The alkylation product compound **17**, was brominated to furnish compound **18**. Compound **18** was subjected to halogen-metal exchange and the resulting species was trapped with 3-nitro-benzoyl chloride to afford compound **19**. Amination of **19** followed with nitro reduction furnished the penultimate amine intermediate **20**.

The final derivatization step, usually formation of sulfonamide from sulfonyl chloride and urea from isocyanates using the penultimate intermediates, was typically carried out in parallel format. Final analogs were prepared in 15–25 mg quantity all purified via reversed phase semi preparative HPLC. We instituted an intuitive



Scheme 1. Synthesis of compound **15**. Reagents and conditions: (a) *i*-PrI, Cs_2CO_3, DMF, rt, 5 h, 94%; (b) NBS, DCM, rt, 12 h, 90%; (c) *n*-BuLi, ether, −78 °C, 0.5 h then Weinreb amide, 73%; (d) dioxane, NH_4OH (1:1), 45 °C, 90%; (e) Fe, NH_4Cl, dioxane/EtOH/H_2O, 100 °C, 80–100% (2 steps); (f) *p*-Cl-Ph-NCO, 80 °C, 68%.

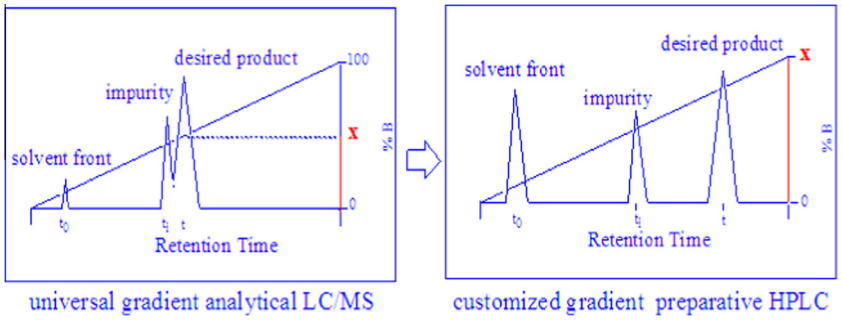


Figure 6. Analytical LCMS—semipreparative HPLC purification paradigm.

paradigm to effectively leverage the analytical LCMS to customize gradient windows for each HPLC purification run for maximal resolution (Fig. 6).[19] For example, if the desired product retention time *t* on the analytical LCMS corresponded to *x*% B on the gradient chart, we configured our systems such that the desired product usually eluted off the column during the semipreparative purification within a gradient window of 5 to *x*% B. This paradigm was deployed routinely and handle the bulk of our samples. For example, dozens of analogs were made with N5-cyclohexyl tail on which further substitutions resulted in cis- and trans-isomers (as in compound **2**). These isomeric mixtures were routinely separated using this paradigm.

In summary, we have established several novel pyrrolopyrimidine series of Tie-2 inhibitors. The ketophenyl urea analogs are reversible and ATP competitive Tie-2 inhibitors with potent activity in whole-cell assays. Medicinal chemistry efforts culminated in the identification of compound **15**, which demonstrated exquisite potency against Tie-2/Trk, and robust dose-responsive oral efficacy. Subsequent efforts to refine attributes of pharmacokinetics, hERG/iKr, and other parameters resulted in a compound advanced into human clinical trial studies.[20]

Acknowledgments

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10. Details of the Tie-2 kinase and cellular assays are disclosed in Ref. 18. (a) Briefly the kinase ELISA assay used a GST-Tie-2 kinase fusion protein and poly-Glu-Tyr coated 96-well plates. Reactions were performed in the presence of 100 μM ATP and phosphorylated products were detected with a horseradish peroxidase-conjugated monoclonal antibody to phosphotyrosine (HRP-PY20). For compound reversibility studies the enzyme was pre-incubated with compound for 15 min before the addition of ATP. Enzyme kinetics was determined by carrying out the above procedure using titrated ATP dilutions. (b) The cellular assay used NIH/3T3 fibroblasts stably transfected with a chimeric receptor composed of the extracellular domain of human EGFR and intracellular domain of human Tie-2. The cells were incubated with compounds for 60 min and then stimulated with EGF before being fixed to the plate. Phosphotyrosine was detected using an Eu-labeled antibody and DELFIA detection reagents. For washout experiments the compound containing media was removed and replaced with fresh media. At indicated time points the cells were stimulated and the assay carried out as described above.
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15. The observed disconnect between the kinase and cellular IC_{50}s is believed to be in part due to the artifacts of kinase constructs and screens. Cellular IC_{50}s may be a more accurate measure of selectivity. This will be discussed in greater details in Ref. 20.
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17. Ex vivo ELISA: 5×10^6 C6 rat glioma cells stably expressing human Tie-2 were injected with 50% matrigel s.c. into the right flank of each mouse. Treatment was initiated when tumors in all mice in each experiment ranged in size from 250 to 350 mm^3. Tumors and plasma were harvested at indicated time points following a single oral dose. Tumors were frozen in liquid nitrogen and subsequently homogenized in lysis buffer and the lysates applied to antibody coated plates. Detection was carried out with HRP-labeled antibodies. Plasma and tumor concentrations were determined by LC–MS using established procedures.
18. (a) Details of step (c): *n*-BuLi (23 mL, 2.5 M in Hexane, 57.3 mmol) was added dropwise to a solution of 5-bromo-4-chloro-7-isopropyl-7*H*-pyrrolo[2,3-d]pyrimidine (15.0 g, 54.64 mmol) in ether (1.2 L) at −78 °C. After 1 h a solution of *N*-methoxy-*N*-methyl-3-nitrobenzamide (15 g, 71.03 mmol) in ether (100 mL) was added by drop wise addition. After 1 h the reaction was quenched with saturated aqueous NH_4Cl and warmed to room temperature. The layers were separated and the aqueous layer was extracted with EtOAc (3×200 mL). The organic layers were combined and washed with water (one time) and brine (one time), then dried over Na_2SO_4 and concentrated. Purification by flash column chromatography (hexanes/ethyl acetate 75:25) afforded the desired product as a white solid (15.21 g, 73%). LRMS: *m*/*z* 345.2 [M+H], $C_{16}H_{13}ClN_4O_3$ calculated 344. ^1H NMR (400 MHz, DMSO-d_6, δ): 1.45 (d, $J = 6.6$, 6H), 5.12–5.05 (m, 1H), 7.84 (t, $J = 7.89$, 1 H), 8.26 (d, $J = 7.5$, 1H), 8.48–8.53 (m, 3H), 8.78 (s, 1H). Additional experimental details on the synthesis of these analogs can be found in the various published patent applications: Acari, J.; Chen, J.; LaGreca, S.; Marx, M. A.; Wessel, M. WO 2004056830.; (b) Wessel, M. D.; Chen, J.; Marx, M. A.; Lagreca, S. D. WO 2005047289 A1.; (c) Marx, M. A.; La Greca, S. D.; Chen, J.; Wessel, M. D.; Arcari, J.T. WO 2005116035 A1.
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20. Optimization of compound **15** into a human clinical trial phase I compound is the subject of a future communication.