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Analogues of 4-[(7-Bromo-2-methyl-4-oxo-3*H*-quinazolin-6-yl)methylprop-2-ynylamino]-*N*-(3-pyridylmethyl)benzamide (CB-30865) as Potent Inhibitors of Nicotinamide Phosphoribosyltransferase (Nampt)

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We have shown previously that the target of the potent cytotoxic agent 4-[(7-bromo-2-methyl-4-oxo-3*H*-quinazolin-6-yl)methyl-prop-2-ynylamino]-*N*-(3-pyridylmethyl)benzamide (CB38065, **1**) is nicotinamide phosphoribosyltransferase (Nampt). With its cellular target known we sought to optimize the biochemical and cellular Nampt activity of **1** as well as its cytotoxicity. It was found that a 3-pyridylmethylamide substituent in the A region was critical to cellular Nampt activity and cytotoxicity, although other aromatic substitution did yield compounds with submicromolar enzymatic inhibition. Small unsaturated groups worked best in the D-region of the molecule, with 3,3-dimethylallyl providing optimal potency. The E region required a quinazolin-4-one or 1,2,3-benzotriazin-4-one group for activity, and many substituents were tolerated at C^2 of the quinazolin-4-one. The best compounds showed subnanomolar inhibition of Nampt and low nanomolar cytotoxicity in cellular assays.

Introduction

The quinazolinone cytotoxic agent CB30865 (**1**, Figure 1a) was developed as part of an effort to improve folate-based inhibitors of thymidylate synthase (TS[a]) by replacing the classical antifolate terminal glutamic acid residue with aminomethylpyridine moieties.[1] The stated goal of this replacement was to increase the lipophilicity and consequently the cell permeability of these compounds and to prevent undesirable intracellular polyglutamation. The 2-, 3-, and 4-pyridylmethylamides were moderately potent inhibitors of mammalian TS (IC_{50} = 156−508 nM), but the 3-aminomethylpyridyl analogue (**1**) showed dramatically increased W1L2 cytotoxicity (EC_{50} = 2.8 nM) that was not rescued by precursors or end-products of folate metabolism.[2] These data and the cytotoxicty profile of **1** across a wide range of cell lines suggested a folate-independent mechanism of action. Water-soluble analogues of **1** were made in an effort to improve in vivo efficacy, and the best of these compounds showed subnanomolar W1L2 cytotoxicity (**2**, EC_{50} = 0.49 nM).[3]

We have previously reported the direct target affinity purification (DTAP) of solid-phase linked analogues of **2**.[4] These chemical proteomics-based studies revealed that the target of **1** and **2** is nicotinamide phosphoribosyltransferase (Nampt, NAMPRTase, EC 2.4.2.12). Compound **2** inhibits Nampt in an enzymatic assay with an average IC_{50} value of 11 nM.[4] Mechanism-based cellular assays confirmed the cellular inhibition of Nampt by **2**: compound **2** causes cellular nicotinamide adenine dinucleotide (NAD) levels to drop, as measured directly and by concomitant loss of poly(ADP-ribose) polymerase (PARP) activity. The drop in cellular NAD levels, PARP inhibition, and the cytotoxicity caused by **2** are completely rescued by the addition of exogenous nicotinic acid, further supporting Nampt inhibition as the mechanism of action of **2**.

Nampt catalyzes the first and rate-limiting step in the conversion of nicotinamide (NAM) to NAD (Figure 2). This salvage pathway is the only way a mammalian cell can recycle the NAM formed during NAD consumption by enzymes such as PARPs and sirtuins.[5] Nampt is critical to cell viability, as NAD is an essential cofactor in ATP generating processes. Cancer cells have both increased demand for ATP and increased activity of NAD-consuming enzymes, and consequently Nampt has emerged as an attractive target for the development of anticancer therapeutics.[6] Phase I clinical results have been reported for two Nampt inhibitors (FK866, **3**, and GMX1778/CHS-828, **4**),[7b] and phase II studies on each are ongoing. More recently 1,2,3-triazole-containing compounds have been reported to be potent Nampt inhibitors: compound **5** depletes cellular NAD levels with IC_{50} = 3.0 nM and shows an SH-SY5Y cytotoxicity of 3.8 nM.[8]

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[a] Abbreviations: TS, thymidylate synthase; DTAP, direct target affinity purification; Nampt, nicotinamide phosphoribosyltransferase; NAD, nicotinamide adenine dinucleotide; PARP, poly(ADP-ribose) polymerase; NAM, nicotinamide; ATP, adenosine triphosphate; NMN, nicotinamde mononucleotide; Naprt, nicotinic acid phosphoribosyltransferase; DHFR, dihydrofolate reductase; NBS, *N*-bromosuccinimide; AIBN, 2,2′-azobis(2-methylpropionitrile); DIC, *N*,*N*′-diisopropylcarbodiimide; HOBt, 1-hydroxybenzotriazole; DPPA, diphenylphosphorylazide; TEPP, tetraethyl pyrophosphate.

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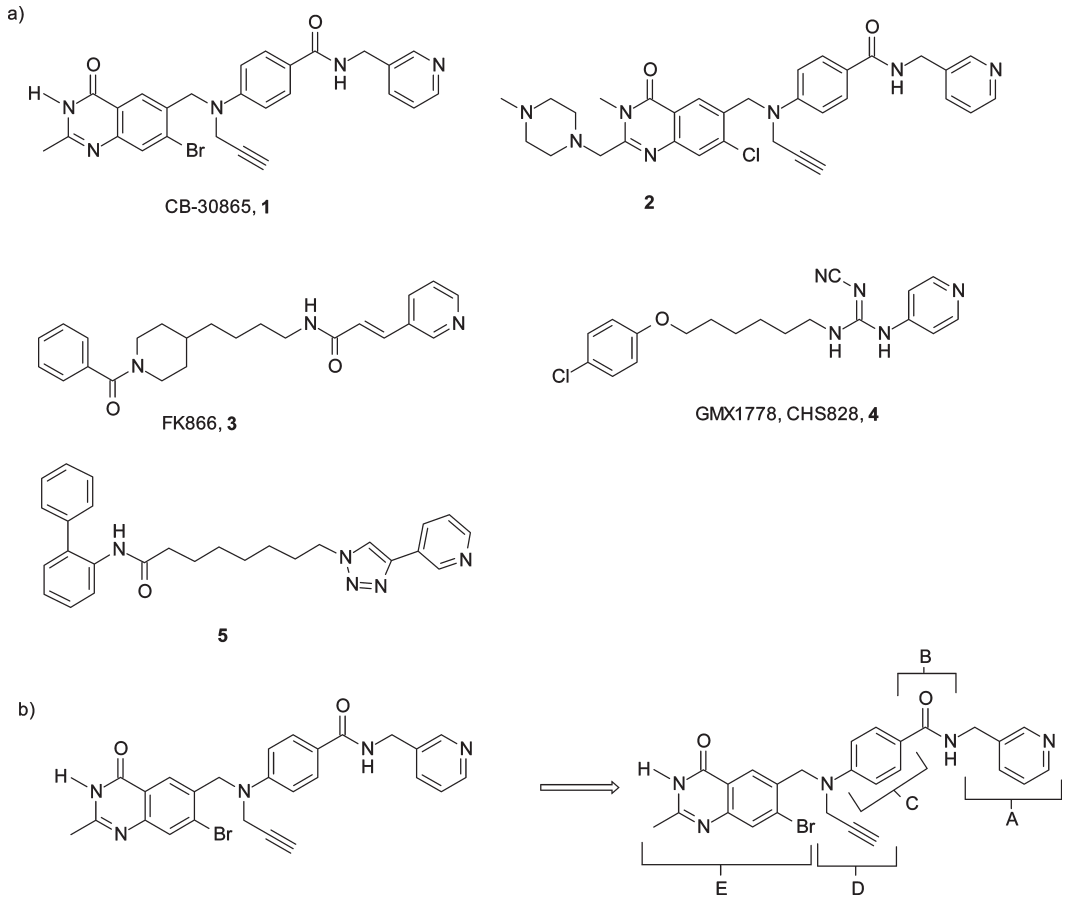


Figure 1. (a) Previously reported Nampt inhibitors. (b) Division of **1** for study.

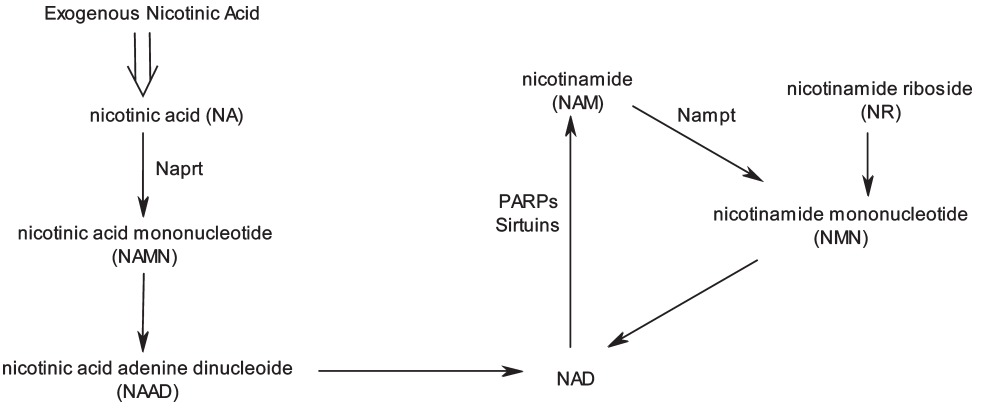


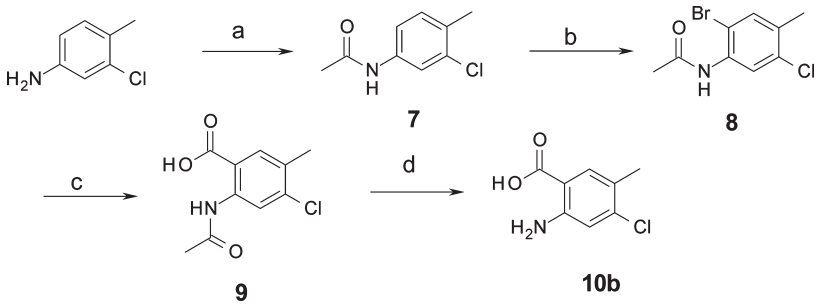
Figure 2. Role of Nampt in the maintanance of cellular NAD levels. Nampt acts as the rate-limiting step in the recycling of NAM formed by NAD consumption by PARPs and sirtuins. When Nampt is inactivated, NAD can be generated in cells expressing Naprt via a compensatory pathway starting with exogenous nicotinic acid. The figure is adapted from ref 5c.

With the enzymatic target of **1** and its water-soluble analogue **2** identified, we undertook efforts to optimize their activity toward Nampt inhibition and cytotoxicity. We divided compound **2** into five structural regions (Figure 1b). The cocrystal structure of **3** in Nampt defined the binding site as roughly cylindrical, with the nicotinamide-mimicking pyridyl moeity buried inside and the distal E-region solvent exposed.[9] The narrow shape of this cavity precluded much SAR in the central portion of the inhibitor, and consequently we focused our efforts on the A, D, and E regions. Thymidylate synthase inhibition is known to be abrogated by alkylation at N^3 of the quinazolinone,[3] and we consequently sought to retain the N^3-methyl group of **2** as much as possible.

Chemistry

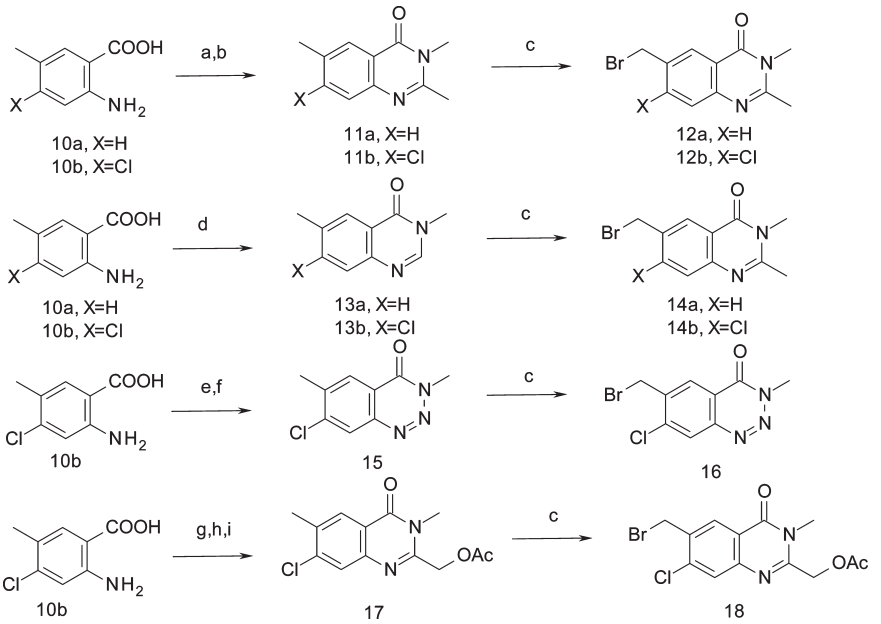
The previously reported synthesis of compound **2**[3] was optimized to allow for the large scale (> 100 g) synthesis of key intermediate 5-methyl-6-chloroanthranilic acid (**10b**, Scheme 1). The protection of 4-methyl-5-chloroaniline with acetic anhydride in EtOAc/pyridine was followed by electrophilic bromine/acetic acid bromination. Lithium−halogen exchange followed by carbon dioxide quench and deprotection led to anthranilic

Scheme 1[a]



[a] Reagents: (a) Ac_2O, pyr, EtOAc; (b) Br_2, AcOH; (c) (i) *n*-BuLi, hexanes/THF, (ii) CO_2 (g); (d) NaOH, H_2O.

Scheme 2[a]



[a] Reagents: (a) Ac_2O; (b) $MeNH_2$−HCl, DMF; (c) NBS, AIBN or $(PhCOO)_2$, CCl_4, *hv*; (d) HCONHMe; (e) $MeNH_2$−HCl, DIC, HOBt, DIEA, DCM; (f) $NaNO_2$, HCl, H_2O; (g) Na, EtOH, $ClCH_2CN$; (h) KOAc, DMF; (i) NaH, MeI, DMF.

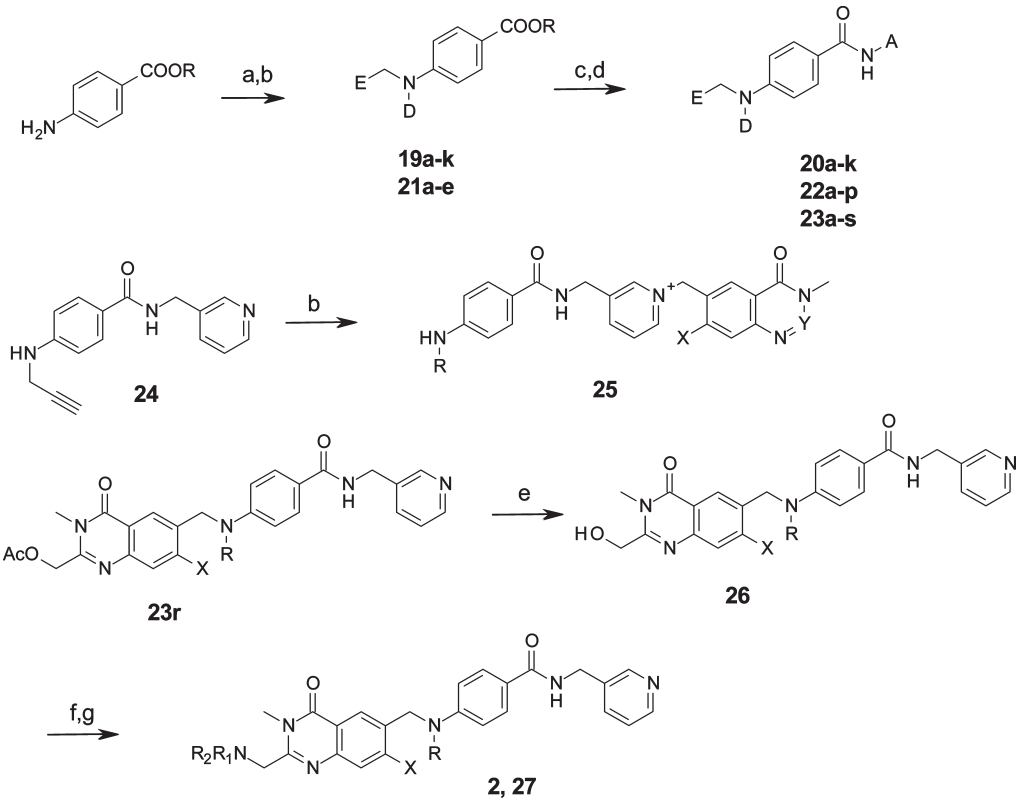
acid **10b**. This carboxylation protocol was found to be much higher yielding than the cyanation−hydrolysis procedure described previously.[3]

Quinazolin-4-one and 1,2,3-benzotriazin-4-one syntheses (Scheme 2) each began with commercially available 5-methylanthranilic acid (**10a**) or 4-chloro-5-methylanthranilic acid **10b**. For 2-methylquinazolin-4-ones **11a** and **11b**, anthranilic acid **10a** or **10b** was heated at reflux in acetic anhydride followed by treatment of the resulting 2-methyl-3,1-benzoxazin-4-one with *N*-methylamine hydrochloride in DMF. Anthranilic acid **10a** or **10b** was heated neat in *N*-methylformamide[10] to yield quinazolinones **13a** and **13b**. Anthranilic acid **10b** was allowed to react with *N*-methylamine hydrochloride in the presence of DIC/HOBt/DIEA/DCM and the resulting anthranilamide treated with sodium nitrite to give 1,2,3-benzotriazin-4-one **15**.[11] Bromination with NBS using catalytic AIBN or dibenzoyl peroxide in carbon tetrachloride led to bromomethyl compounds **12a**/**b**, **14a**/**b**, and **16**. Irradiation of the reaction mixture with UV light was found to be critical to this bromination. The synthesis of methylene-linked E regions proceeded largely as described in the literature.[3] Anthranilic acid **10b** was treated with sodium metal and chloroacetonitrile to generate 7-chloro-2-(chloromethyl)-6-methyl-3*H*-quinazolin-4-one, and chloride displacement with potassium acetate followed by NaH/iodomethane methylation yielded ester **17**. We found that esterification with potassium acetate proceeded in much higher yield than described previously[3] with cesium acetate. The quinazolin-4-one ester **17** was brominated analogously to **11a**/**b** to yield bromide **18**.

Esters of 4-aminobenzoic acid were alkylated with the appropriate D-region bromide in the presence of 2,6-lutidine in DMF followed by reaction with 2-chlorobenzyl bromide or bromomethyl heterocycle **12**, **14**, **16**, or **18** (Scheme 3). Amides were made using the appropriate acid chloride and Et_3N in DCM followed by alkylation as described above. Esters **19a**−**k** and **21a**−**e** were deprotected and coupled with 3-aminomethylpyridine in the presence of DIC/HOBt/DIEA in DCM to yield **20a**−**k**, **22a**−**p**, and **23a**−**s**. It was found that if amide coupling preceded the addition of the bromomethyl heterocycle, alkylation occurred on the pyridyl nitrogen of **24** to yield quaternary pyridinium salt **25** rather than the desired N-alkylated aniline. These salts were found to be inactive against Nampt.

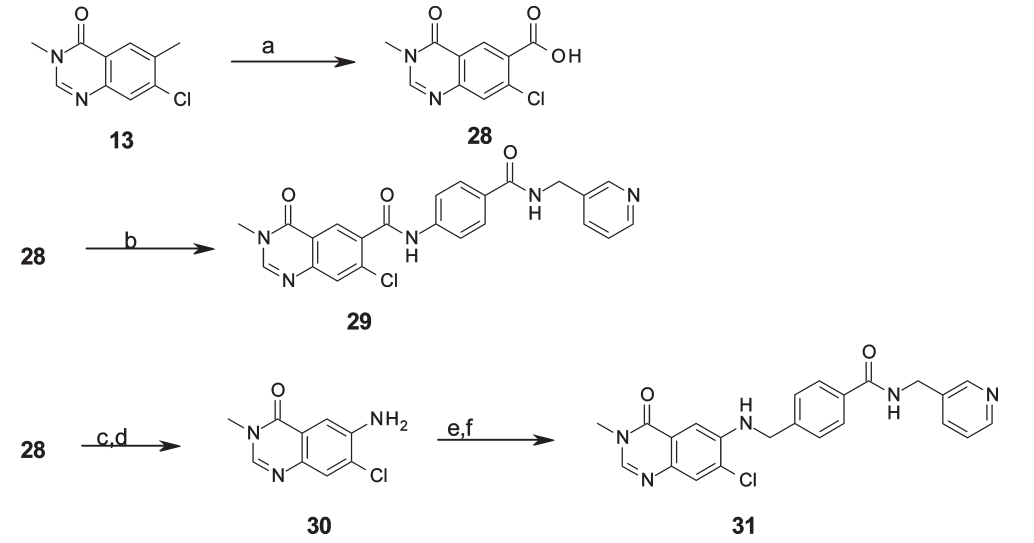
Functionalization at the C^2 methylene of ester **23r** also necessitated changes to literature procedures (Scheme 3). Whereas hydrolysis of acetate **23r** was previously described using aqueous sodium hydroxide, in our hands these conditions

Scheme 3[a]



[a] See Tables 1−3 for A, D, and E substituents. Reagents: (a) D-Br, 2,6-lutidine, DMF or RCOCl, Et_3N, DCM; (b) E-Br (2-chlorobenzyl bromide, **12**, **14**, **16**, or **18**), 2,6-lutidine, DMF; (c) NaOH, EtOH/H_2O or TFA/DCM; (d) A-NH_2, DIC, HOBt, DIEA, DCM; (e) H_2SO_4, MeOH; (f) Ms_2O, Et_3N, DCM; (g) HNR_1R_2.

Scheme 4[a]

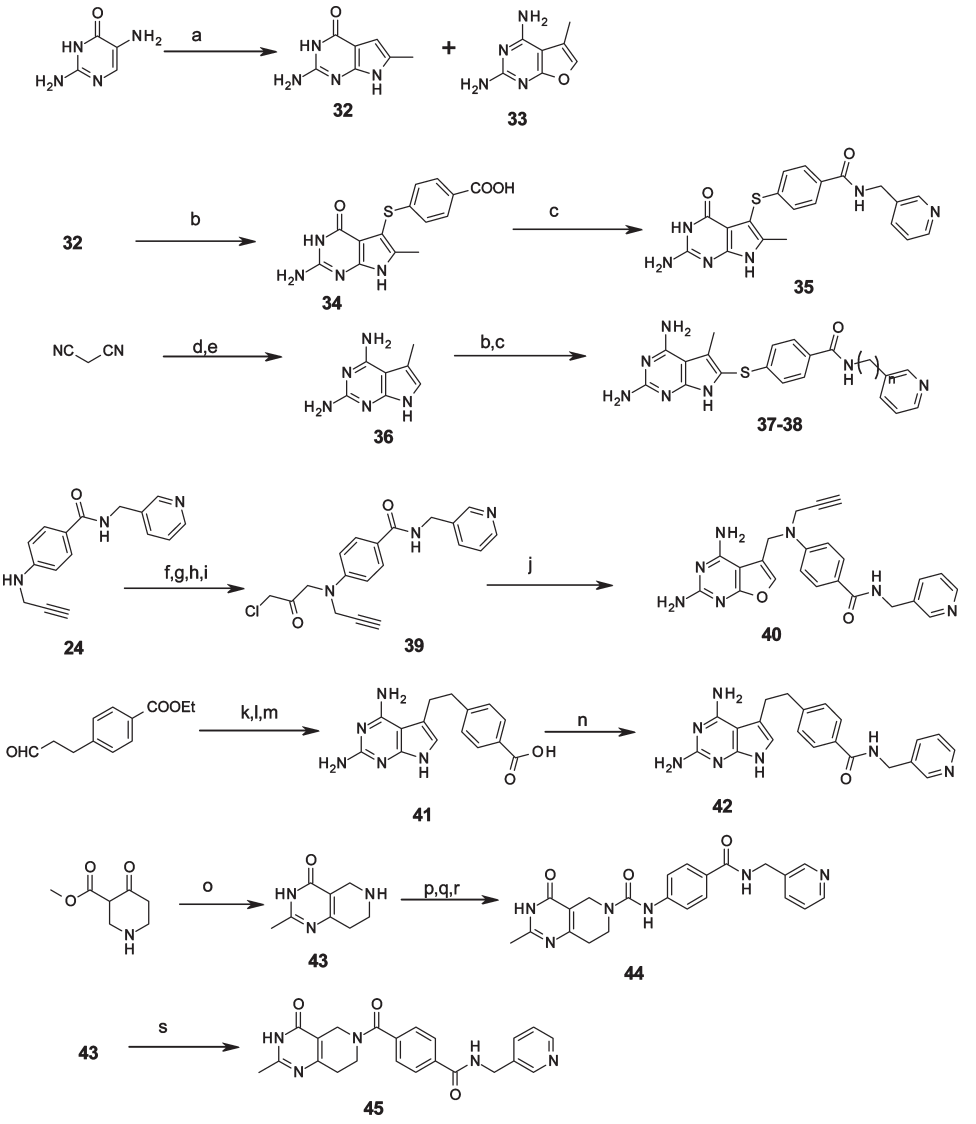


[a] Reagents: (a) $KMnO_4$, pyr/H_2O; (b) 4-amino-*N*-(3-pyridylmethyl)benzamide, TEPP, $PhCH_3$; (c) DPPA, Et_3N, *t*-BuOH; (d) TFA, DCM; (e) 4-carboxybenzoic acid, $NaBH(OAc)_3$, TFA, *i*-PrOAc; (f) 3-aminomethylpyridine, DIC, HOBt, DIEA, DCM.

resulted in the opening of the quinazolinone ring, yielding an acylated anthranilic acid derivative. Acidic hydrolysis of the acetate was successfully performed with H_2SO_4/MeOH. Alcohol **26** was converted into the methanesulfonate ester with Ms_2O and displaced with amines to yield C^2 aminomethyl compounds **2** and **27**.

Additional changes to the D-region of the molecule were achieved by oxidation of 7-chloro-3,6-dimethylquinazolin-4-one (**13**) to carboxylic acid **28** with potassium permanganate (Scheme 4). Standard carbodiimide coupling of acid **28** to the 3-aminomethylpyridylamide of 4-aminobenzoic acid was unsuccessful, and consequently TEPP was utilized to produce amide **29**. Coupling with secondary anilines such as **24** were also unsuccessful regardless of coupling agent employed. To generate the reverse amine linkage in the D-region, carboxylic acid **28** was allowed to react with diphenylphosphorylazide

Scheme 5[a]



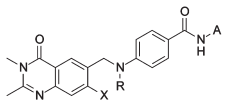
[a] Conditions: (a) chloroacetone, DMF; (b) I_2, 4-mercaptobenzoic acid, EtOH/H_2O; (c) RNH_2, DIC, HOBt, DIEA, DCM; (d) hydroxyacetone, Et_3N, MeOH; (e) guanidine−HCl, NaOEt/EtOH; (f) *tert*-butyl bromoacetate, 2,6-lutidine, DMF; (g) TFA/DCM; (h) $(COCl)_2$, DCM/DMF; (i) (i) $TMSCHN_2$, Et_2O, (ii) HCl (g); (j) 2,6-diamino-4-pyrimidone, DMF; (k) paraformaldehyde, 3-ethylthiazoline bromide, Et_3N, EtOH; (l) malononitrile, Et_3N, MeOH; (m) guanidine−HCl, KOEt, EtOH; (n) 3-aminomethylpyridine, DIC, HOBt, DIEA, DMF; (o) guanidine−HCl, Na, EtOH; (p) ethyl 4-isocyanatobenzoate, DCM; (q) LiOH, H_2O; (r) 3-aminomethylpyridine, EDCI, HOBt, Et_3N, DMF; (s) 4-(3-pyridylmethylcarbamoyl)benzoic acid, EDCI, HOBt, Et_3N, DMF.

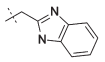
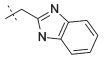
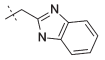
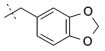
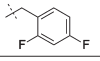
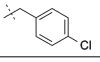
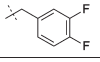
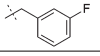
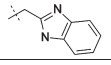
(DPPA) in *tert*-butanol under Curtius conditions[12] and the resulting Boc-protected amine deprotected with TFA. Reductive amination[13] of 5-aminoquinazolinone **30** with 4-formylbenzoic acid followed by DIC/HOBt coupling with 3-aminomethylpyridine led to reverse amine **31**.

More dramatic changes to the quinazolinone core were made using literature methods reported for the synthesis of thymidylate synthase and dihydrofolate reductase inhibitors (Scheme 5). The condensation of 2,6-diamino-4-pyrimidone with chloroacetone generated a mixture of 2-amino-4-oxo-6-methylpyrrolo[2,3,*d*]pyrimidine (**32**) and 5-methylfuro[2,3-*d*]pyrimidine-2,4-diamine (**33**).[14b] The reaction of heterocycle **32** with iodine and 4-mercaptobenzoic acid followed by DIC/HOBt/DIEA-mediated amidation with 3-aminomethylpyridine yielded compound **35**.[14] Malononitrile was condensed with hydroxyacetone followed by guanidine hydrochloride to generate 2,4-diamino-5-methylpyrrolo[2,3-*d*]pyrimidine (**36**), which was then allowed to react with iodine and 4-mercaptobenzoic acid followed by DIC/HOBt/DIEA-mediated amidation to give compounds **37** and **38**.[15]

Aniline **24** was alkylated with *tert*-butyl bromoacetate, deprotected with TFA/DCM, and the resulting acid was converted into the corresponding acid chloride via oxalyl chloride activation. A diazoketone was generated in situ through reaction with TMS-CHN_2 and quenched with HCl gas to yield α-chloroketone **39**.[17] α-Chloroketone **39** underwent ring closure with 2,6-diamino-4-pyrimidone to yield furo[2,3-*d*]-pyrimidine **40**.[14] The expected side product of 2-amino-4-oxo-5-alkylated-pyrrolo[2,3,*d*]pyrimidine was not isolated. Ethyl 4-(3-oxopropyl)benzoate was treated with paraformaldehyde in the presence of Et_3N and 3-ethylbenzothiazoline bromide and the resulting α-hydroxy ketone condensed with malononitrile. Ring closure with guanidine hydrochloride and concomitant ester hydrolysis produced acid **41**. This acid was

Table 1. A-Region Substitution[a]



Cmpd	X	R	A	IC50 (μM)	Cytotox (μM)	PARP (μM)
22a	Cl	P		0.040	5.7	3.2
22b	Cl	P		0.00055	0.0021	0.00045
22c	Cl	D		0.011	0.0095	0.0018
22d	Cl	D		0.025	6.4	>5
22e	H	P		0.0036	0.034	0.0095
22f	H	P		0.13	5.4	>5
22g	H	P		>10	n/d	n/d
22h	H	P		0.085	>10	>5
22i	H	P		0.034	9.7	>5
22j	H	P		0.049	>10	>5
22k	H	P		0.098	>10	>5
22l	H	P		0.15	>10	>5
22m	H	P		0.030	>10	>5
22n	H	P		0.25	>10	>5
22o	H	D		0.0014	0.0035	0.00023
22p	H	D		0.031	1.4	0.7

[a] R = P: propargyl. R = D: 3,3-dimethylallyl. n/d = not done.

coupled with 3-aminomethylpyridine in the presence of DIC/HOBt/DIEA to generate compound **42**.[16]

Lastly, 5,6,7,8-tetrahydro-3*H*-pyrido[4,3-*d*]pyrimidin-4-one core **43** was made through the condensation of ethyl 4-oxopiperidine-3-carboxylate with guanidine hydrochloride in the presence of NaOEt/EtOH. Urea formation with heterocycle **43** and ethyl 4-isocyanatobenzoate followed by ester hydrolysis with lithium hydroxide and peptide coupling with 3-aminomethylpyridine yielded **44**. Amidation of **43** with 4-(3-pyridylmethylcarbamoyl)benzoic acid produced compound **45**.

Biological Evaluation and Discussion

Compounds were screened in a coupled-enzyme assay that monitored the Nampt-catalyzed conversion of NAM to NMN.[4] Cytotoxicity was determined by monitoring ATP levels after 72 h of treatment of HCT116 colon carcinoma cells. Cellular inhibition of Nampt was measured by the loss of PARP activity after H_2O_2-induced DNA damage in MCF-10A cells stably transduced with the PIK3CA(H1047R) oncogene.[4] Cytotoxicity in the presence of 10 μM exogenous nicotinic acid was measured in selected compounds to confirm Nampt inhibition as the basis of cell killing.

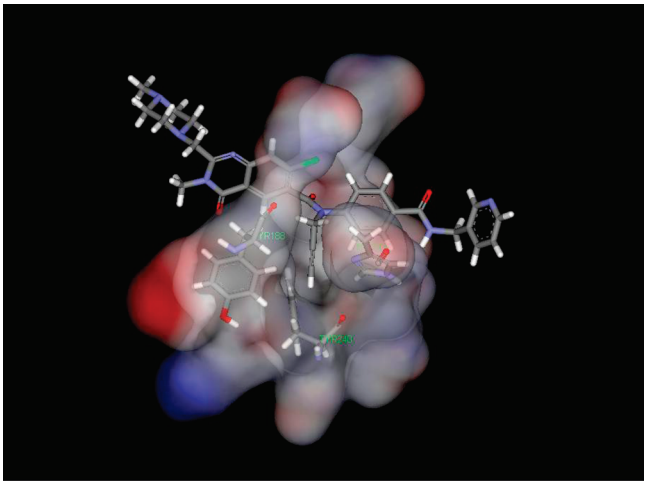


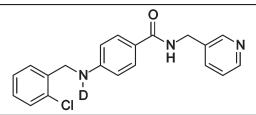
Figure 3. Compound **2** docked into Nampt active site. The D-region propargyl group is positioned in a cleft bordered by Tyr188, His191, and Tyr240.

The cocrystal structure of an analogue of **3** with Nampt[9b] shows its pyridyl ring in the substrate binding pocket where it is capable of interacting with the protein in two possible ways: a salt bridge can be formed between the pyridyl nitrogen and the acidic hydrogens of Asp219 or Asp16, and the pyridyl ring is positioned between Phe193 and Tyr18 which could allow for a π-stacking interaction. To gauge the importance of these interactions, the A-region was substituted with a variety of aromatic, heteroaromatic, and aliphatic substituents.

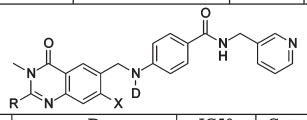
The A-region was found to be rather intolerant of substitution, as only the 3-pyridyl moiety of **1** was found to give subnanomolar Nampt inhibition or low nanomolar 3-day HCT116 cytotoxicity (Table 1). Moderate activity was observed with a 1*H*-benzimidazol-2-ylmethylamide, but other basic functionalities such as aliphatic and anilinic amines (exemplified by **22g**) showed complete inactivity at 10 μM. Inhibition was seen with a benzimidazole A-region regardless of the rest of the molecule. The activity of benzimidazole derivatives **22a**, **22d**, **22f**, and **22p** was comparable to that of the 2-pyridyl analogue of **2** (IC_{50} = 170 nM),[4] suggesting that while a 3-pyridyl substituent is optimally situated to participate in a salt bridge with Asp16 or Asp219 a basic heteroaromatic group positioned differently can also participate but to a lesser degree. Weak biochemical activity was also seen with several A-region aromatic groups. Electron-deficient rings were generally more potent than electron-rich systems, supporting the involvement of π-stacking interactions, although 3,4-methylenedioxy substitution (**22h**) also showed good inhibition. The importance of π-stacking is also suggested by the complete inactivity of 3-piperidyl analogue **22g**.

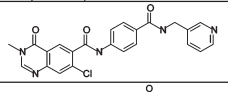
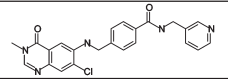
It is noteworthy that while the 2-pyridyl analogue of **1** showed cytotoxicty commensurate with its biochemical inhibition,[4] all other analogues in this series with similar inhibitory activities were almost completely inactive in cells. Preliminary data show that while these compounds inhibited Nampt with submicromolar potencies, the maximum inhibition of Nampt was approximately 90% compared to that of untreated enzyme (data not shown). Compounds that exhibit cytotoxicity and cellular Nampt inhibition typically showed biochemical Nampt inhibition of 95−96%. Additional studies are underway to

Table 2. D-Region Substitution[a]



Cmpd	D	IC50 (μM)	Cytotox (μM)	PARP (μM)
20a		>10	n/d	n/d
20b		0.33	>10	>5
20c		0.015	3.5	0.24
20d		>10	n/d	n/d
20e		0.42	n/d	n/d
20f		0.0061	0.39	0.0079
20g		0.0042	2.4	0.15
20h		0.0013	0.059	0.011
20i		0.0019	0.016	0.0032
20j		0.0015	0.099	0.0089
20k		0.11	0.7	0.17



Cmpd	R	X	D	IC50 (μM)	Cytotox (μM)	PARP (μM)
23a	H	H		0.012	0.3	0.025
23b	H	H		0.0012	0.004	0.0004
23c	H	Cl		0.0026	0.0073	0.0005
23d	H	Cl		0.013	0.11	0.012
23e	H	Cl		0.056	>10	>1
23f	H	Cl		0.012	0.15	0.03
23g	H	Cl		0.66	4.1	0.37
23h	CH_3	H		0.21	5.3	0.012
23i	CH_3	H		0.0029	0.0044	0.0016
23j	CH_3	H		0.95	6.3	2.9
23k	CH_3	H		2.6	0.11	0.019
23l	CH_3	H		>10	>10	2.6
23m	CH_3	H		>10	>10	2.2
29				0.012	0.73	0.048
31				0.0038	0.42	0.0093

[a] n/d = not done.

investigate the connection between this higher maximum inhibitory effect and cellular activity.

Molecular modeling of **2** suggested that the D-region substituent binds into a cleft defined by Tyr188, His191, and Tyr240 of the Nampt peptide (Figure 3). To explore the nature of this cleft, compounds with varying D-regions were synthesized and screened (Table 2). For initial D-region exploration a synthetically simplified series was constructed wherein the quinazolinone portion of **2** was replaced by a 2-chlorophenyl group. These compounds generally showed significantly reduced Nampt inhibition and cytotoxicity compared to the quinazolinone series. Straight-chain aliphatic groups increased in activity according to their size up to C_3 (Me $\ll$ Et $<$ Pr $\gg$ Bu), and branching was tolerated at C^3 but not C^2. Substitution by unsaturated groups containing three to four carbons was found to be ideal, and a 3,3-dimethylallyl moiety was the most potent compound tested in this series (**20i**, HCT116 EC_{50} = 16 nM). Benzyl substitution at this position (**20k**) was shown to be too large, giving a 10-fold reduction in activity compared to the most active substituents in the biochemical, cellular, and cytotoxicity assays.

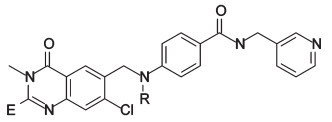
The best of these groups was then synthesized within the quinazolinone series. As with the simplified system, 3,3-dimethylallyl (**22c**, **22o**, **23b**, **23c**) showed the strongest inhibitory activities across all quinazolinone series with the best compounds showing cytotoxicity EC_{50} values less than 10 nM. In addition propargyl substitution (**22b**, **22e**, **23a**) yielded compounds with activity similar to that of 3,3-dimethylallyl. Modeling suggested that the polarizable electrons of the unsaturated system could interact in an edge-to-face fashion with all three aromatic residues bordering the binding pocket (Tyr188, His191, and Tyr240), leading to increased activity over aliphatic groups of similar length (Figure 3). An alternative explanation for the activity of propargyl and 3,3-dimethylallyl analogues is that the cleft is simply too narrow to accommodate a sp^3-hybridized carbon past the β position relative to the nitrogen while a more flat sp^2 or sp system fills the cavity without steric clashes.

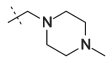
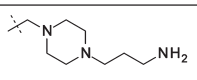
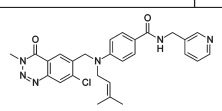
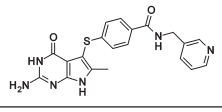
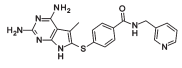
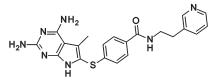
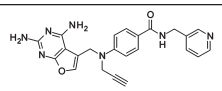
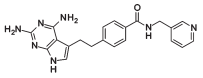
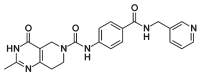
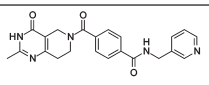
To distinguish between these possibilities, additional analogues were synthesized. The most success was found with methylcyclopropyl (**23d**, **23k**) and homopropargyl (**23f**, **23j**) substitution, while methylacetamide (**23e**) and 3,5-dimethyl-4-isoxazole (**23h**) substitution gave compounds with moderate activity. Compound **23h** showed no cytotoxicity despite sub-100 nM potency against the enzyme. The biochemical activity of **23h** despite its considerable size downplays the potential involvement of sterics, although its lack of cytotoxicity suggests that sterics play some role. Nitrogen acylation led to inactive compounds, where modeling predicted an unfavorable steric clash at the D-region side chain α-position. Lastly, more radical changes were undertaken in the D-region. Reversing the benzylamine group (**31**) resulted in moderate inhibitory activity, as did replacement of the benzylamine linkage with a benzamide (**29**). These compounds did show cytotoxicity commensurate with their biochemical activities.

Variation around the E-region of **1** led to extremely potent compounds when a heteroaromatic system with two six-membered rings was retained (Table 3). Addition of the 3-aminopropyl linker to **2** led to a 40-fold reduction in cytotoxicity (**27**); however, much of the activity was regained by replacement of the *N*-methylpiperazine moiety with either a hydroxyl group (**26**) or a proton (**22b**). The lower than expected cytotoxicity of **27** compared to its biochemical potency was likely the result of its high polarity and concomitant low cellular permeability. Much less variation in activity was demonstrated in the 3,3-dimethylallyl series, as low nanomolar cytotoxicities were observed with 2-unsubstituted and 2-methyl analogues (**22c**, **23c**), as well as with the 1,2,3-triazolinone (**22q**).

As more dramatic changes to the quinazolinone core are known to be tolerated in both inhibition of TS and DHFR,[13−15] several other heterocycles were synthesized and screened against Nampt. While a number of these compounds showed submicromolar activity against Nampt in the biochemical assay, none showed significant cellular Nampt activity or cytotoxicty at 10 μM.

Table 3. E-Region Substitution[a]



Cmpd	E	R[a]	IC50 (μM)	Cytotox (μM)	PARP (μM)
2		P	0.00031	0.00046	0.00005
26		P	0.00079	0.0016	0.0001
27		P	0.00030	0.018	0.0018
22q			0.00045	0.003	0.0017
35			2.8	n/d	>5
37			1.2	n/d	n/d
38			0.090	>10	n/d
40			>10	>10	>5
42			0.60	>10	>5
44			0.029	>10	>5
45			7.2	>10	>5

[a] R = P: propargyl. R = D: 3,3-dimethylallyl. n/d = not done.

In all compounds tested the mechanism-based cellular PARP assay results were consistent with both Nampt inhibition and HCT116 cytotoxicity. Compounds with A-region variation that possess biochemical potency but lack HCT116 cytotoxicity show a similar inactivity in the PARP assay. Lastly, the cytoxicity of several potent analogues (**20f**, **20g**, **20i**, **22c**, **23c**, and **23e**) was rescued (EC_{50} > 10 μM) when tested in the presence of 10 μM exogenous nicotinic acid. These data strongly suggest that Nampt inhibition was the primary cause of their cytotoxicity.

In conclusion, a series of Nampt inhibitors was designed and synthesized based on the quinazolin-4-one **1** and its water-soluble analogue **2**. A 3-substituted pyridine group in the A-region was required for significant HCT116 cytotoxicity, although weakly potent Nampt inhibition was achieved with a variety of aromatic substitution. A small unsaturated group in the D-region was optimal for both Nampt inhibition and cytotoxicity. Polarizable electrons that can interact in an edge-to-face manner with Tyr188, His191, or Tyr240 of the Nampt protein are implicated by modeling to be the cause of this preference, although simple sterics cannot be discounted. A quinazolin-4-one or 1,2,3-benzotriazin-4-one is required for activity in the E-region of the molecule, although a variety of substituents at the C^2 position are tolerated. Several Nampt inhibitors with low nanomolar biochemical, cellular, and cytotoxicity potencies were synthesized. Studies are underway to further characterize their cellular and in vivo effects.

Experimental Section

General Methods and Materials. The ^1H NMR spectra were recorded at 400 MHz. Chemical shifts are reported in parts per million (ppm) downfield from TMS (0.00 ppm), and *J* coupling constants are reported in hertz. HPLC−MS experiments were run in the ESI mode using an Xterra MS C18 (Waters) 4.6 mm × 50 mm, 5 μm column. HPLC purity was determined using a 4.6 mm ×150 mm Xterra C18 5 μm column, observing at both 203 and 280 nm. Both HPLC−MS and HPLC were reverse phase with an AcCN/H_2O (0.01% v/v TFA) gradient and a flow rate of 0.5 mL/min. All final compounds were ≥95% pure by HPLC at both 203 and 280 nm. MPLC purifications were performed using an Isco RF system utilizing either a hexane/EtOAc or DCM/MeOH gradient. The synthesis of compounds **2** and **27** has been previously described.[4]

***N*-(2-Bromo-5-chloro-4-methylphenyl)acetamide[3] (8).** Acetic anhydride (142 mL, 1.5 mol, 1.5 equiv) is added slowly to 3-chloro-4-methylaniline (142 g, 1 mol) and pyridine (120 mL, 1.5 mol, 1.5 equiv) in 1 L of ethyl acetate at 0 °C. The solution was allowed to warm to room temperature over 90 min and washed with 4 × 200 mL of 10% aqueous HCl. The organic layer was dried with Na_2SO_4 and concentrated. Acetic acid (1 L) was added and the solution fitted with a mechanical stirrer. Bromine (96 mL, 1.9 mol, 1.9 equiv) in 100 mL of acetic acid was added dropwise at 0 °C and the solution allowed to warm to room temperature overnight. The suspension was filtered and the filtrate concentrated. DCM (1 L) was added to the resulting solid, and $NaHCO_3$ was added until the solid dissolved. The solution was washed with water, 10% $Na_2S_2O_3$, dried with Na_2SO_4, and concentrated to approximately 300 mL. The resulting suspension was filtered and the filtrate washed with saturated $NaHCO_3$, dried with Na_2SO_4, and concentrated to approximately 100 mL. The resulting suspension was filtered again and solid combined with the first fraction of solid. The combined solids were dried under vacuum to yield 221 g of white solid (84%). The solid was carried on without further purification.

2-Amino-4-chloro-5-methylbenzoic Acid[3] (10b). *n*-BuLi (2.5 M in hexanes, 440 mmol, 2.2 equiv) is added dropwise at −78 °C over 2.5 h to bromide **8** (52.5 g, 200 mmol) in 1 L of THF. The solution is stirred at −78 °C for 15 min, and CO_2 is bubbled through the solution as it warmed from −78 °C to room temperature overnight. A small amount of MeOH is added and the solution concentrated. DCM (600 mL) is added and the solution washed with 10% aqueous HCl, dried with Na_2SO_4, and concentrated. The resulting solid is triturated with DCM to yield 24.32 g of white solid (9, 54%). A 5 M solution of sodium hydroxide (110 mL, 5 equiv) is added and the reaction heated at reflux overnight. The solution is cooled, and concentrated HCl is added to obtain pH 4−5. The resulting solid is filtered and dried under vacuum and 17.5 g of white solid isolated (87%).

2,3,6-Trimethylquinazolin-4-one (11a). Acetic anhydride (0.5 mL/mmol) is added to 5-methylanthranilic acid and the suspension heated at reflux for 1 h, cooled to room temperature, and filtered. The solid is triturated with Et_2O. Methylamine hydrochloride (3 equiv) and DMF (1 M) are added, and the solution is heated at 120 °C overnight. Ethyl acetate is added, washed with saturated $NaHCO_3$, dried with Na_2SO_4, and concentrated. **11b** is synthesized analogously from **8**.

6-(Bromomethyl)-2,3-dimethylquinazolin-4-one (12a). Quinazoline **11**, NBS (1.1 equiv), and AIBN (5 mol %) in CCl_4 (0.2 M) are heated at reflux under a UV lamp until reaction is complete by LC/MS. The solution is washed with 10% aqueous sodium bisulfite, dried with Na_2SO_4, and concentrated. The resulting

solid is purified by MPLC (0−100% EtOAc/hexanes). **12b** is made analogously from **11b**.

3,6-Dimethylquinazolin-4-one (13a). 5-Methylanthranilic acid is heated in *N*-methylformamide (4 equiv) at 180 °C for 2 h. The solution is cooled, diluted with EtOAc, washed with 1 M NaOH, dried with Na_2SO_4, and concentrated. **13b** is synthesized analogously from **8**.

6-(Bromomethyl)-3-methylquinazolin-4-one (14a). Quinazolinones **13a** and **13b** are brominated analogously to **11a**.

3,6-Dimethyl-7-chloro-1,2,3-benzotriazin-4-one (15). DIEA (3 equiv) is added to **8** (1 equiv), methylamine hydrochloride (1.2 equiv), EDCI (1.2 equiv), and HOBt (1.2 equiv) in DCM and the solution stirred at room temperature overnight. The solution is concentrated and purified by MPLC (0−20% MeOH/DCM). The resulting solid is suspended in 3.6 M HCl and $NaNO_2/H_2O$ (1.1 equiv, 2 M) is added dropwise at 0 °C. The solution is warmed from 0 °C to room temperature over 2 h, and 3 mL of 10 N NaOH is added. Concentrated HCl is added to obtain pH 2 and the solution cooled at 0 °C. The resulting solid is filtered and dried under vacuum.

6-(Bromomethyl)-3-methyl-1,2,3-benzotriazin-4-one (16). Quinazolinone **15** is brominated analogously to **11**.

General Procedure for the Synthesis of 20, 22, and 23. Alkyl bromide (1.2 equiv) is added to ethyl 4-aminobenzoate or *tert*-butyl 4-aminobenzoate (1 equiv) and 2,6-lutidine (1.2 equiv) in DMF (0.5 M) and the solution heated at 70 °C overnight. The solution is concentrated and purified by MPLC (0−60% EtOAc/hex).

Bromide **12**, **14**, **16**, or **18** (1 equiv) is added to N-alkylated 4-aminobenzoate ester (1 equiv) and 2,6-lutidine (1.2 equiv) in DMF (0.5 M) and the solution heated at 70 °C overnight. The solution is concentrated and purified by MPLC (0−60% EtOAc/hex). Ethyl esters are cleaved by adding NaOH (2.5 equiv) in 1/1 $EtOH/H_2O$ and heating at reflux for 1−2 h. The solution is cooled, acidified with 10% HCl, and washed with EtOAc. The organic layer is dried with Na_2SO_4, filtered, and concentrated. *tert*-Butyl esters are added to ∼5 mL/mmol 1:1 TFA/DCM and stirred at room temperature for 30−90 min and concentrated. The resulting acids are dissolved in DCM, and 3-aminomethylpyridine (1.2 equiv), DIC (1.2 equiv), HOBt (1.2 equiv) are added followed by DIEA (3 equiv). The solution is stirred at room temperature overnight, concentrated, and purified by MPLC (0−15% MeOH/DCM).

4-[(2-Chlorobenzyl)(methyl)amino]-*N*-(pyridin-3-ylmethyl)-benzamide (20a). ^{1}H NMR (DMSO-d_6) δ: 8.69−8.62 (m, 2H), 8.12 (bs, 1H), 7.73 (t, J = 6.0 Hz, 1H), 7.44−7.32 (m, 5H), 7.30 (d, J = 9.0 Hz, 2H), 6.52 (d, J = 9.0 Hz, 2H), 4.70 (s, 4H), 2.69 (s, 3H). HRMS [M + H] calculated for $C_{21}H_{20}ClN_3O$ 366.136 77, found 366.136 83.

4-[(2-Chlorobenzyl)(ethyl)amino]-*N*-(pyridin-3-ylmethyl)benzamide (20b). ^{1}H NMR (DMSO-d_6) δ: 8.80 (t, J = 5.6 Hz, 1H), 8.69 (d, J = 2.0 Hz, 1H), 8.63 (dd, J = 1.2, 8.4 Hz, 1H), 8.09 d, J = 8.0 Hz, 1H), 7.72−7.68 (m, 3H), 7.50 (dd, 1.6, 8.0 Hz, 1H), 7.32−7.24 (m, 2H), 7.03 (dd, J = 2.0, 7.6 Hz, 1H), 6.62 (d, 9.6, 2H), 4.62 (s, 2H), 4.52 (d, J = 5.6 Hz, 2H), 3.55 (q, 7.2 Hz, 2H), 1.17 (t, J = 6.8 Hz, 3H). HRMS [M + H] calculated for $C_{22}H_{22}ClN_3O$ 380.1524, found 380.149 92.

4-[(2-Chlorobenzyl)(propyl)amino]-*N*-(pyridin-3-ylmethyl)-benzamide (20c). ^{1}H NMR (DMSO-d_6) δ: 8.72 (t, J = 6.4 Hz, 1H), 8.53 (s, 1H), 8.46 (dd, J = 1.2, 4.8 Hz, 1H), 7.73 (dt, J = 8.0, 2.0 Hz, 1H), 7.69 (d, J = 9.2 Hz, 2H), 7.50 (dd, J = 1.6, 8.0 Hz, 1H), 7.38 (ddd, J = 0.8, 4.0, 7.6 Hz, 1H), 7.31−7.22 (m, 2H), 7.00 (dd, J = 2.0, 7.6 Hz, 1H), 6.60 (d, J = 9.2 Hz, 2H), 4.65 (s, 2H), 4.45 (d, J = 5.6 Hz, 2H), 3.44 (t, J = 7.6 Hz, 2H), 1.68−1.58 (m, 2H), 0.91 (t, J = 7.2 Hz, 3H). HRMS [M + H] calculated for $C_{23}H_{24}Cl\ N_3O$ 394.168 07, found 394.171 49.

4-[Butyl(2-chlorobenzyl)amino]-*N*-(pyridin-3-ylmethyl)benzamide (20d). ^{1}H NMR (DMSO-d_6) δ: 8.73 (d, J = 4.3 Hz, 1H), 8.70 (s, 1H), 7.85 (dd, J = 7.8, 5.5 Hz, 1H), 7.42−7.30 (m, 5H), 7.27 (d, J = 8.6 Hz, 2H), 6.54 (d, J = 8.6 Hz, 2H), 4.72 (s, 4H), 3.01 (t, J = 7.0 Hz, 2H), 1.55−1.46 (m, 2H), 1.41−1.30 (m, 2H), 0.90 (t, J = 7.4 Hz, 3H). HRMS [M + H] calculated for $C_{24}H_{26}ClN_3O$ 408.183 72, found 408.187 43.

4-[(2-Chlorobenzyl)(2-methylpropyl)amino]-*N*-(pyridin-3-ylmethyl)benzamide (20e). ^{1}H NMR (DMSO-d_6) δ: 8.77 (t, J = 6.0 Hz, 1H), 8.68 (br s, 1H), 8.63 (d, J = 4.8 Hz, 1H), 8.07 (d, J = 8.0 Hz, 1H), 7.70−7.67 (m, 3H), 7.49 (dd, J = 1.6, 8.0 Hz, 1H), 7.30−7.22 (m, 2H), 6.96 (dd, J = 1.6, 7.6 Hz, 1H), 6.63 (d, J = 9.2 Hz, 2H), 4.69 (s, 2H), 4.51 (d, J = 6.0 Hz, 2H), 3.35 (d, J = 7.6 Hz, 2H), 2.13−2.06 (m, 1H), 0.95 (s, 3H), 0.93 (s, 3H). HRMS [M + H] calculated for $C_{24}H_{26}ClN_3O$ 408.183 72, found 408.183 42.

4-[(2-Chlorobenzyl)(3-methylbutyl)amino]-*N*-(pyridin-3-ylmethyl)benzamide (20f). ^{1}H NMR (DMSO-d_6) δ: 8.78 (t, J = 6.0 Hz, 1H), 8.66 (d, J = 2.0 Hz, 1H), 8.60 (dd, J = 1.6, 5.6 Hz, 1H), 8.04 (dt, J = 8.4, 2.0 Hz, 1H), 7.70 (d, J = 9.2 Hz, 2H), 7.65 (dd, J = 5.2, 8.0 Hz, 1H), 7.50 (dd, J = 1.6, 8.0 Hz, 1H), 7.32−7.24 (m, 2H), 7.03 (dd, J = 2.0, 7.6 Hz, 1H), 6.59 (d, J = 8.8 Hz, 2H), 4.63 (s, 2H), 4.51 (d, J = 6.0 Hz, 2H), 3.48 (t, J = 7.6 Hz, 2H), 1.67−1.58 (m, 1H), 1.50 (q, J = 6.8 Hz, 2H), 0.93 (s, 3H), 0.91 (s, 3H). HRMS [M + H] calculated for $C_{25}H_{28}ClN_3O$ 422.199 37, found 422.200 43.

4-[(2-Chlorobenzyl)(prop-2-yn-1-yl)amino]-*N*-(pyridin-3-ylmethyl)benzamide (20g). ^{1}H NMR (DMSO-d_6) δ: 8.86 (t, J = 5.9 Hz, 1H), 8.63 (d, J = 15.0 Hz, 2H), 8.00 (d, J = 6.8 Hz, 1H), 7.76 (d, J = 9.0 Hz, 2H), 7.61 (dd, J = 7.8, 5.1 Hz, 1H), 7.51 (d, J = 7.4 Hz, 1H), 7.34−7.24 (m, 2H), 7.18 (d, J = 7.0 Hz, 1H), 6.75 (d, J = 9.0 Hz, 2H), 4.71 (s, 3H), 4.52 (d, J = 5.9 Hz, 2H), 4.35 (d, J = 2.0 Hz, 2H), 3.26 (t, J = 2.0 Hz, 1H). HRMS [M + H] calculated for $C_{23}H_{20}ClN_3O$ 390.1375, found 390.1697.

4-[(2-Chlorobenzyl)(cyclopropylmethyl)amino]-*N*-(pyridin-3-ylmethyl)benzamide (20h). ^{1}H NMR (DMSO-d_6) δ: 8.79 (t, J = 6.0 Hz, 1H), 8.68 (d, J = 1.6 Hz, 1H), 8.63 (dd, J = 1.2, 5.2 Hz, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.71−7.67 (m, 3H), 7.48 (dd, J = 1.6, 7.6 Hz, 1H), 7.30−7.21 (m, 2H), 7.06 (dd, J = 1.6, 7.6 Hz, 1H), 6.66 (d, J = 8.8 Hz, 2H), 4.72 (s, 2H), 4.51 (d, J = 5.6 Hz, 2H), 3.43 (d, J = 6.4 Hz, 2H), 1.5−1.08 (m, 1H), 0.48−.043 (m, 2H), 0.28−.024 (m, 2H). HRMS [M + H] calculated for $C_{24}H_{24}ClN_3O$ 406.168 07, found 406.167 98.

4-[(2-Chlorobenzyl)(3-methylbut-2-en-1-yl)amino]-*N*-(pyridin-3-ylmethyl)benzamide (20i). ^{1}H NMR (DMSO-d_6) δ: 8.73 (t, J = 6.0 Hz, 1H), 8.55 (d, J = 1.2 Hz, 1H), 8.48 (dd, J = 1.2, 3.6 Hz, 1H), 7.77 (dt, J = 6.4, 2.0 Hz, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.48 (dd, J = 1.6, 4.4 Hz, 1H), 7.42 (dd, J = 4.4, 6.4 Hz, 1H), 7.31−7.24 (m, 2H), 7.09 (dd, J = 2.0, 6.4 Hz, 1H), 6.62 (d, J = 7.2 Hz, 2H), 5.26−5.23 (m, 1H), 4.61 (s, 2H), 4.45 (d, J = 4.8 Hz, 2H), 4.07 (d, J = 6.4 Hz, 2H), 1.69 (s, 3H), 1.65 (s, 3H). HRMS [M + H] calculated for $C_{25}H_{26}ClN_3O$ [M + H] 420.183 72, found 420.185 09.

4-[(2*E*)-But-2-en-1-yl(2-chlorobenzyl)amino]-*N*-(pyridin-3-ylmethyl)benzamide (20j). ^{1}H NMR (DMSO-d_6) δ: 8.79 (t, J = 5.6 Hz, 1H), 8.67 (d, J = 1.2 Hz, 1H), 8.62 (dd, 1.2, 5.2 Hz, 1H), 8.06 (d, J = 7.6 Hz, 1H), 7.70 −7.64 (m, 3H), 7.50 (dd, J = 2.0, 8.0 Hz, 1H), 7.32−7.25 (m, 2H), 7.05 (dd, J = 2.0, 7.2 Hz, 1H), 6.63 (d, J = 9.6 Hz, 2H), 5.66−5.50 (m, 2H), 4.61 (s, 2H), 4.51 (d, J = 6.0 Hz, 2H), 4.05 (d, J = 5.2 Hz, 2H), 1.65 (dd, J = 1.2, 6.4 Hz, 3H). HRMS [M + H] calculated for $C_{24}H_{24}ClN_3O$ 406.168 07, found 406.168 44.

4-[Benzyl(2-chlorobenzyl)amino]-*N*-(pyridin-3-ylmethyl)benzamide (20k). ^{1}H NMR (DMSO-d_6) δ: 8.77 (t, J = 5.6 Hz, 1H), 8.65 (br s, 1H), 8.60 (d, J = 4.8 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.68−7.62 (m, 3H), 7.50 (dd, J = 2.4, 6.8 Hz, 1H), 7.36−7.23 (m, 7 H), 7.12 (dd, J = 2.8, 6.4 Hz, 1H), 6.64 (d, J = 9.2 Hz, 2H), 4.79 (d, J = 6.8 Hz, 4H), 4.49 (d, J = 6.0 Hz, 2H). HRMS [M + H] calculated for $C_{27}H_{24}ClN_3O$ 442.168 07, found 442.167 12.

***N*-(1*H*-Benzimidazol-2-ylmethyl)-4-{[(7-chloro-2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](prop-2-yn-1-yl)amino}-benzamide (22a).** ^{1}H NMR (DMSO-d_6) δ: 7.87 (s, 1H), 7.82 (d, J = 9.0 Hz, 2H), 7.75 (s, 1H), 7.74−7.66 (m, 2H), 7.49−7.42 (m, 2H), 6.84 (d, J = 9.0 Hz, 2H), 4.83 (d, J = 5.1 Hz, 2H), 4.79 (s, 2H), 4.41 (d, J = 2.0 Hz, 2H), 3.47 (s, 3H), 3.24 (t, J = 2.0 Hz, 1H),

2.56 (s, 3H). HRMS [M + H] calculated for $C_{29}H_{25}ClN_6O_2$ 525.180 03, found 525.178 71.

4-{[(7-Chloro-2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)-methyl](prop-2-yn-1-yl)amino}-*N*-(pyridin-3-ylmethyl)benzamide (22b). ^{1}H NMR (DMSO-d_6) δ: 8.88 (t, J = 5.8 Hz, 1H), 8.66 (s, 1H), 8.59 (d, J = 5.1 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.88 (s, 1H), 7.76 (d, J = 9.0 Hz, 2H), 7.74 (s, 1H), 7.62 (dd, J = 7.8, 5.1 Hz, 1H), 6.79 (d, J = 9.0 Hz, 2H), 4.77 (s, 2H), 4.52 (d, J = 5.8 Hz, 2H), 4.38 (d, J = 2.3 Hz, 2H), 3.46 (s, 3H), 3.24 (t, J = 2.3 Hz, 1H), 2.56 (s, 3H). HRMS [M + H] calculated for $C_{27}H_{24}ClN_5O_2$ 486.169 13, found 486.167 90.

4-{[(7-Chloro-2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)-methyl](3-methylbut-2-en-1-yl)amino}-*N*-(pyridin-3-ylmethyl)-benzamide (22c). ^{1}H NMR (DMSO-d_6) δ: 8.76 (t, J = 5.8 Hz, 1H), 8.59 (d, J = 1.5 Hz, 1H), 8.53 (dd, J = 3.5, 1.5 Hz, 1H), 8.09 (s, 1H), 7.86 (d, J = 7.8 Hz, 1H), 7.70 (d, J = 9.0 Hz, 2H), 7.51 (s, 1H), 7.48 (d, J = 8.5 Hz, 1H), 6.78 (d, J = 9.0 Hz, 2H), 5.30 (t, J = 6.2 Hz, 1H), 4.78 (s, 2H), 4.48 (d, J = 6.3 Hz, 2H), 4.07 (d, J = 5.8 Hz, 2H), 3.56 (s, 3H), 2.44 (s, 3H), 1.67 (s, 3H), 1.63 (s, 3H). HRMS [M + H] calculated for $C_{29}H_{30}ClN_5O_2$ 516.216 08, found 516.215 77.

***N*-(1*H*-Benzimidazol-2-ylmethyl)-4-{[(7-chloro-2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](3-methylbut-2-en-1-yl)-amino}benzamide (22d).** ^{1}H NMR (DMSO-d_6) δ: 12.13 (s, 1H), 8.79 (t, J = 5.9 Hz,1H), 8.08 (s, 1H), 7.76 (d, J = 9.0 Hz, 2H), 7.52 (s, 1H), 7.42 (d, J = 6.6 Hz, 1H), 7.15−7.08 (m, 2H), 6.80 (d, J = 9.0 Hz, 2H), 5.31 (t, J = 6.4 Hz, 1H), 4.79 (s, 2H), 4.64 (d, J = 5.9 Hz, 2H), 4.09 (d, J = 6.4 Hz, 2H), 3.57 (s, 3H), 2.45 (s, 3H), 1.67 (s, 3H), 1.63 (s, 3H). HRMS [M + H] calculated for $C_{31}H_{31}ClN_6O_2$ 555.226 98, found 555.226 64.

4-{[(2,3-Dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]-(prop-2-yn-1-yl)amino}-*N*-(pyridin-3-ylmethyl)benzamide (22e). ^{1}H NMR (DMSO-d_6) δ: 8.77 (t, J = 5.8 Hz, 1 h), 8.51 (d, J = 1.6 Hz, 1H), 8.43 (dd, J = 5.0, 1.9 Hz, 1H), 8.00 (d, J = 1.6 Hz, 1H), 7.73 (d, J = 9.0 Hz, 2H), 7.69 (tm, J = 7.6 Hz, 1H), 7.55 (d, J = 8.6 Hz, 1H), 7.33 (dd, J = 7.8, 4.7 Hz, 1H), 6.84 (d, J = 9.0 Hz, 2H), 4.78 (s, 2H), 4.45 (d, J = 5.8 Hz, 2H), 4.33 (d, J = 2.4 Hz, 2H), 3.51 (s, 3H), 3.22 (t, J = 2.4 Hz, 1H), 2.56 (s, 3H). HRMS [M + H] calculated for $C_{27}H_{25}N_5O_2$ 452.208 10, found 452.210 90.

***N*-(1*H*-Benzimidazol-2-ylmethyl)-4-{[(2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](prop-2-yn-1-yl)amino}benzamide (22f).** HRMS [M + H] calculated for $C_{29}H_{26}N_6O_2$ 491.2190, found 491.2182.

4-{[(2,3-Dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]-(prop-2-yn-1-yl)amino}-*N*-(piperidin-3-ylmethyl)benzamide (22g). HRMS [M + H] calculated for $C_{27}H_{31}N_5O_2$ 458.255 05, found 458.254 45.

***N*-(1,3-Benzodioxol-5-ylmethyl)-4-{[(2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](prop-2-yn-1-yl)amino}benzamide (22h).** ^{1}H NMR (DMSO-d_6) δ: 8.66 (t, J = 6.0 Hz, 1H), 8.00 (d, J = 2.0 Hz, 1H), 7.73 (d, J = 9.0 Hz, 2H), 7.70 (dd, J = 8.6, 2.3 Hz, 1H), 7.55 (d, J = 8.2 Hz, 1H), 6.85−6.81 (m, 4H), 6.75 (dd, J = 7.8, 1.5 Hz, 1H), 5.99 (s, 2H), 4.79 (s, 2H), 4.36−4.30 (m, 4H), 3.51 (s, 3H), 3.23 (t, J = 2.0, 1H), 2.56 (s, 3H). HRMS [M + H] calculated for $C_{29}H_{26}N_4O_4$ 495.202 68, found 495.202 14.

4-{[(2,3-Dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]-(prop-2-yn-1-yl)amino}-*N*-(2-fluorobenzyl)benzamide (22i). ^{1}H NMR (DMSO-d_6) δ: 8.71 (t, J = 6.0 Hz, 1H), 8.01 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 9.0 Hz, 2H), 7.72 (dd, J = 8.6, 1.6 Hz, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.34−7.25 (m, 2H), 7.15 (q, J = 7.4 Hz, 2H), 6.84 (d, J = 9.0 Hz, 2H), 4.80 (s, 2H), 4.47 (d, J = 5.8 Hz, 2H), 4.35 (d, J = 2.0 Hz, 2H), 3.52 (s, 3H), 3.24 (t, J = 2.0 Hz, 1H), 2.58 (s, 3H). HRMS [M + H] calculated for $C_{28}H_{25}FN_4O_2$ 469.203 43, found 429.202 11.

***N*-(2,4-Difluorobenzyl)-4-{[(2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](prop-2-yn-1-yl)amino}benzamide (22j).** ^{1}H NMR (DMSO-d_6) δ: 8.71 (t, J = 5.9 Hz, 1H), 8.00 (d, J = 1.6 Hz, 1H), 7.74 (d, J = 9.0 Hz, 2H), 7.70 (dd, J = 8.6, 2.0 Hz, 1H), 7.56 (d, J = 8.6 Hz, 1H), 7.35 (q, J = 6.6 Hz, 1H), 7.21 (qd, J = 9.4, 2.3 Hz, 1H), 7.03 (td, J = 7.8, 2.3 Hz, 1H), 6.83 (d, J = 9.0 Hz, 2H), 4.80 (s, 2H), 4.43 (d, J = 5.5 Hz, 2H), 4.34 (d, J = 2.3 Hz, 2H), 3.57 (s, 3H), 3.24 (t, J = 2.3 Hz, 1H), 2.57 (s, 3H). HRMS [M + H] calculated for $C_{28}H_{24}F_2N_4O_2$ 487.194 01, found 487.192 88.

4-{[(2,3-Dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]-(prop-2-yn-1-yl)amino}-*N*-(2,4,5-trifluorobenzyl)benzamide (22k). ^{1}H NMR (DMSO-d_6) δ: 8.73 (t, J = 5.9 Hz, 1H), 8.01 (d, J = 1.6 Hz, 1H), 7.74 (d, J = 9.0 Hz, 2H), 7.72 (dd, J = 8.7, 2.0 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.55−7.50 (m, 1H), 7.40−7.32 (m, 1H), 6.83 (d, J = 9.3 Hz, 2H), 4.80 (s, 2H), 4.41 (d, J = 5.5 Hz, 2H), 4.35 (d, J = 2.4 Hz, 2H), 3.51 (s, 3H), 3.24 (t, J = 2.4 Hz, 1H), 2.58 (s, 3H). HRMS [M + H] calculated for $C_{28}H_{23}F_3N_4$ O2 505.184 59, found 505.184 11.

***N*-(4-Chlorobenzyl)-4-{[(2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](prop-2-yn-1-yl)amino}benzamide (22l).** HRMS [M + H] calculated for $C_{28}H_{25}ClN_4O_2$ 485.173 88, found 485.173 31.

***N*-(3,4-Difluorobenzyl)-4-{[(2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](prop-2-yn-1-yl)amino}benzamide (22m).** ^{1}H NMR (DMSO-d_6) δ: 8.77 (t, J = 6.2 Hz, 1H), 8.00 (d, J = 2.0 Hz, 1H), 7.74 (d, J = 9.4 Hz, 2H), 7.71 (dd, J = 8.2, 2.0 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.38−7.27 (m, 2H), 7.14−7.09 (m, 1H), 6.84 (d, J = 9.0 Hz, 2H), 4.79 (s, 2H), 4.41 (d, J = 6.2 Hz, 2H), 4.34 (d, J = 2.3 Hz, 2H), 3.51 (s, 3H), 3.24 (t, J = 2.3 Hz, 1H), 2.57 (s, 3H). HRMS [M + H] calculated for $C_{28}H_{24}F_2N_4O_2$ 487.194 01, found 487.194 85.

4-{[(2,3-Dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]-(prop-2-yn-1-yl)amino}-*N*-(3-fluorobenzyl)benzamide (22n). ^{1}H NMR (DMSO-d_6) δ: 8.77 (t, J = 6.2 Hz, 1H), 8.00 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 9.0 Hz, 2H), 7.70 (dd, J = 6.6, 2.0 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.38−7.31 (m, 1H), 7.14−7.01 (m, 3H), 6.84 (d, J = 9.4 Hz, 2H), 4.79 (s, 2H), 4.44 (d, J = 5.9 Hz, 2H), 4.34 (d, J = 2.4 Hz, 2H), 3.51 (s, 3H), 3.24 (t, J = 2.0, 1H), 2.56 (s, 3H). HRMS [M + H] calculated for $C_{28}H_{25}FN_4O_2$ 469.203 43, found 469.202 76.

4-{[(2,3-Dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]-(3-methylbut-2-en-1-yl)amino}-*N*-(pyridin-3-ylmethyl)benzamide (22o). ^{1}H NMR (DMSO-d_6) δ: 8.80 (t, J = 5.2 Hz, 1H), 8.70 (d, J = 2.8 Hz, 1H), 8.65 (d, J = 2.5 Hz, 1H), 8.12 (bs, 1H), 7.91 (d, J = 1.6 Hz, 1H), 7.69 (d, J = 9.4 Hz, 2H), 7.66 (dd, J = 8.2, 2.3 Hz, 1H), 7.56 (d, J = 8.6 Hz, 1H), 6.71 (d, J = 9.0 Hz, 2H), 5.22 (t, J = 6.2 Hz, 1H), 4.73 (s, 2H), 4.52 (d, J = 5.5 Hz, 2H), 4.10 (d, J = 6.8 Hz, 2H), 3.51 (s, 3H), 2.57 (s, 3H), 1.68 (s, 3H), 1.66 (s, 3H). HRMS [M + H] calculated for $C_{29}H_{31}N_5O_2$ 482.255 05, found 482.257 55.

***N*-(1*H*-Benzimidazol-2-ylmethyl)-4-{[(2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](3-methylbut-2-en-1-yl)amino}-benzamide (22p).** ^{1}H NMR (DMSO-d_6) δ: 9.04 (t, J = 5.1 Hz, 1H), 7.90 (d, J = 1.5 Hz, 1H), 7.77−7.70 (m, 4H), 7.65 (dd, J = 8.6, 1.9 Hz, 1H), 7.57 (d, J = 8.6 Hz, 1H), 7.52−7.47 (m, 2H), 6.76 (d, J = 9.4 Hz, 2H), 5.23 (t, J = 6.6 Hz, 1H), 4.84 (d, J = 5.1 Hz, 2H), 4.76 (s, 2H), 4.12 (d, J = 6.6 Hz, 2H), 3.51 (s, 3H), 2.56 (s, 3H), 1.69 (s, 3H), 1.67 (s, 3H). HRMS [M + H] calculated for $C_{31}H_{32}N_6O_2$ 521.265 95, found 521.266 21.

4-{[(7-Chloro-3-methyl-4-oxo-3,4-dihydro-1,2,3-benzotriazin-6-yl)methyl](3-methylbut-2-en-1-yl)amino}-*N*-(pyridin-3-ylmethyl)-benzamide (22q). ^{1}H NMR (DMSO-d_6) δ: 8.82 (t, J = 5.9 Hz, 1H), 8.65 (d, J = 2.1 Hz, 1H), 8.59 (dd, J = 4.7, 2.1 Hz, 1H), 8.43 (s, 1H), 8.04−7.98 (m, 1H), 7.86 (s, 1H), 7.72 (d, J = 9.0 Hz, 2H), 7.63 (bs, 1H), 6.69 (d, J = 9.4 Hz, 2H), 5.28 (t, J = 6.6 Hz, 1H), 4.79 (d, J = 5.9 Hz, 2H), 4.51 (d, J = 5.9 Hz, 2H), 4.15 (d, J = 6.6 Hz, 2H), 3.87 (s, 3H), 1.69 (s, 3H), 1.66 (s, 3H). HRMS [M + H] calculated for $C_{27}H_{27}ClN_6O_2$ 503.195 68, found 503.196 89.

4-{[(3-Methyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](prop-2-yn-1-yl)amino}-*N*-(pyridin-3-ylmethyl)benzamide (23a). ^{1}H NMR (DMSO-d_6) δ: 8.91 (t, J = 5.9 Hz, 1H), 8.79 (d, J = 1.6 Hz, 1H), 8.74 (dd, J = 4.3, 1.6 Hz, 1H), 8.37 (s, 1H), 8.31 (d, J = 8.2 Hz, 1H), 8.05 (d, J = 2.0 Hz, 1H), 7.88 (dd, J = 7.8, 5.4 Hz, 1H), 7.77−7.72 (m, 3H), 7.65 (d, J = 8.2 Hz, 1H), 6.84 (d, J = 9.0 Hz, 2H), 4.82 (s, 2H), 4.57 (d, J = 5.8 Hz, 2H), 4.36 (d, J = 2.0 Hz, 2H),

3.47 (s, 3H), 3.25 (t, J = 2.0 Hz, 1H). HRMS [M + H] calculated for $C_{26}H_{23}N_5O_2$ 438.192 45, found 438.192 23.

4-{(3-Methylbut-2-en-1-yl)[(3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]amino}-*N*-(pyridin-3-ylmethyl)benzamide (23b). ^{1}H NMR (DMSO-d_6) δ: 8.81 (t, J = 6.8 Hz, 1H), 8.72 (d, J = 1.8 Hz, 1H), 8.67 (d, J = 5.1, 1.8 Hz, 1H), 8.36 (s, 1H), 8.18 (d, J = 8.6 Hz, 1H), 7.97 (d, J = 1.2 Hz, 1H), 7.80−7.75 (m, 1H), 7.72−7.62 (m, 4H), 6.71 (d, J = 9.4 Hz, 2H), 5.23 (t, J = 6.5 Hz, 1H), 4.75 (s, 2H), 4.53 (d, J = 5.8 Hz, 2H), 4.11 (d, J = 6.8 Hz, 2H), 3.47 (s, 3H), 1.69 (s, 3H), 1.67 (s, 3H). HRMS [M + H] calculated for $C_{28}H_{29}N_5O_2$ 468.239 40, found 468.239 86.

4-{[(7-Chloro-3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](3-methylbut-2-en-1-yl)amino}-*N*-(pyridin-3-ylmethyl)benzamide (23c). ^{1}H NMR (DMSO-d_6) δ: 8.80 (t, J = 5.8 Hz, 1H), 8.64 (d, J = 1.5 Hz, 1H), 8.58 (dd, J = 5.1 Hz, 1.4, 1H), 8.39 (s, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 3.1 Hz, 2H), 7.72 (d, J = 9.0 Hz, 2H), 7.60 (dd, J = 7.9, 5.1 Hz, 1H), 6.67 (d, J = 9.0 Hz, 2H), 5.27 (t, J = 6.2 Hz, 1H), 4.71 (s, 2H), 4.50 (d, J = 5.5 Hz, 2H), 4.13 (d, J = 6.2 Hz, 2H), 3.43 (s, 3H), 1.69 (s, 3H), 1.67 (s, 3H). HRMS [M + H] calculated for $C_{28}H_{28}ClN_5O_2$ 502.200 43, found 502.202 60.

4-{[(7-Chloro-3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](cyclopropylmethyl)amino}-*N*-(pyridin-3-ylmethyl)benzamide (23d). ^{1}H NMR (DMSO-d_6) δ: 8.78 (dd, J = 6, 6 Hz, 1H), 8.59 (s, 1H), 8.52 (d, J = 4 Hz, 1H), 8.38 (s, 1H), 7.86 (s, 1H), 7.84 (s, 1H), 7.71 (d, J = 9 Hz, 2H), 7.49 (dd, J = 7, 5, 5 Hz, 1H), 6.71 (d, J = 8 Hz, 2H), 4.82 (s, 2H), 4.47 (d, J = 5 Hz, 2H), 3.49 (d, J = 6 Hz, 2H), 3.41 (s, 3H), 1.20−1.08 (m, 1H), 0.48−0.42 (m, 2H), 0.30−0.25 (m, 2H). HRMS [M + H] calculated for $C_{27}H_{26}ClN_5O_2$ 488.1847, found 488.182 95.

4-{(2-Amino-2-oxoethyl)[(7-chloro-3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]amino}-*N*-(pyridin-3-ylmethyl)benzamide (23e). ^{1}H NMR (DMSO-d_6) δ: 8.87 (dd, J = 5, 5 Hz, 1H), 8.72 (s, 1H), 8.67 (d, J = 5 Hz, 1H), 8.40 (s, 1H), 8.16 (d, J = 7 Hz, 1H), 7.85 (s, 1H), 7.79−7.73 (m, 2H), 7.71 (s, 1H), 7.57 (s, 1H), 7.24 (s, 1H), 6.58 (d, J = 8 Hz, 2H), 4.82 (s, 2H), 4.54 (d, J = 5 Hz, 2H), 4.13 (s, 2H), 3.42 (s, 3H). HRMS [M + H] calculated for $C_{25}H_{23}ClN_6O_3$ 491.159 29, found 491.159 06.

4-{But-3-yn-1-yl[(7-chloro-3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]amino}-*N*-(pyridin-3-ylmethyl)benzamide (23f). ^{1}H NMR (DMSO-d_6) δ: 8.86 (dd, J = 5, 5 Hz, 1H), 8.70 (s, 1H), 8.65 (d, J = 4 Hz, 1H), 8.39 (s, 1H), 8.12 (d, J = 8 Hz, 1H), 7.85 (s, 1H), 7.73 (d, J = 4 Hz, 2 Hz), 7.71 (s, 1H), 6.71 (d, J = 9 Hz, 2H), 4.83 (s, 2H), 4.53 (d, J = 5 Hz, 2H), 3.75 (dd, J = 6, 6 Hz, 2H), 3.42 (s, 3H), 2.92 (d, J = 2, 2 Hz, 1H), 2.59−2.53 (m, 2H). HRMS [M + H] calculated for $C_{27}H_{24}ClN_5O_2$ 486.169 13, found 486.1685.

4-{[(7-Chloro-3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](3-methylbut-2-enoyl)amino}-*N*-(pyridin-3-ylmethyl)benzamide (23g). ^{1}H NMR (DMSO-d_6) δ: 9.21 (t, J = 5.9 Hz, 1H), 8.74 (s, 1H), 8.66 (d, J = 4.3 Hz, 1H), 8.39 (s, 1H), 8.16 (d, J = 7.4 Hz, 1H), 8.07 (s, 1H), 7.85 (d, J = 8.6 Hz, 2H), 7.73 (s, 1H), 7.33 (d, J = 8.6 Hz, 2H), 5.57 (s, 1H), 5.18 (s, 2H), 4.55 (d, J = 5.5 Hz, 2H), 3.46 (s, 3H), 2.07 (s, 3H), 1.70 (s, 3H). HRMS [M + H] calculated for $C_{28}H_{26}ClN_5O_3$ 516.179 69, found 516.182 69.

4-{[(3,5-Dimethyl-1,2-oxazol-4-yl)methyl][(2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]amino}-*N*-(pyridin-3-ylmethyl)-benzamide (23h). ^{1}H NMR (DMSO-d_6) δ: 8.88 (dd, J = 6, 6 Hz, 1H), 8.77 (s, 1H), 8.72 (d, J = 5 Hz, 1H), 8.27 (d, J = 7 Hz, 1H), 7.88−7.81 (m, 2H), 7.71 (d, J = 9 Hz, 1H), 7.63 (dd, J = 8, 1, 1 Hz, 1H), 7.56 (d, J = 8 Hz, 1H), 6.85 (d, J = 8 Hz, 2H), 4.77 (s, 2H), 4.58 (s, 2H), 4.55 (d, J = 5 Hz, 2H), 3.51 (s, 3H), 2.58 (s, 3H), 2.24 (s, 3H), 2.12 (s, 3H). HRMS [M + H] calculated for $C_{30}H_{30}N_6O_3$ 524.229 23, found 524.249 44.

4-{(2*E*)-But-2-en-1-yl[(2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]amino}-*N*-(pyridin-3-ylmethyl)benzamide (23i). ^{1}H NMR (DMSO-d_6) δ: 8.81 (dd, J = 5, 5 Hz, 1H), 8.71 (s, 1H), 8.66 (d, J = 5 Hz, 1H), 8.15 (d, J = 7 Hz, 1H), 7.9 (d, J = 1 Hz, 1H), 7.75 (dd, J = 8, 5, 5 Hz, 1H), 7.72−7.64 (m, 3H), 7.57 (d, J = 8 Hz, 1H), 6.72 (d, J = 9 Hz, 2H), 5.69−5.57 (m, 1H), 4.73 (s, 2H), 4.53 (d, J = 5 Hz, 2H), 4.08 (d, J = 4 Hz, 2H), 3.51 (s, 3H), 2.57 (s, 3H), 1.66 (d, J = 6 Hz, 3H). HRMS [M + H] calculated for $C_{28}H_{29}N_5O_2$ 468.2394, found 468.240 15.

4-{[(2,3-Dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](3-methylbut-2-en-1-yl)amino}-*N*-(pyridin-3-ylmethyl)benzamide (23j). ^{1}H NMR (DMSO-d_6) δ: 9.20 (s, 1H), 9.17 (d, J = 6 Hz, 1H), 8.85 (m, 1H), 8.51 (d, J = 8 Hz, 1H), 8.35 (s, 1H), 8.15 (dd, J = 7, 6, 6 Hz, 1H), 7.93 (dd, J = 8, 2, 2 Hz, 1H), 7.68−7.56 (m, 3H), 6.56 (d, J = 9 Hz, 2H), 5.99 (s, 2H), 5.24 (m, 1H), 4.59 (d, J = 5 Hz, 2H), 3.66 (d, J = 6 Hz, 1H), 3.54 (s, 3H), 2.60 (s, 3H), 2.0 (s, 3H), 1.70 (d, J = 5 Hz, 3H). HRMS [M + H] calculated for $C_{29}H_{31}N_5O_2$ 482.255 05, found 482.261 05.

4-{(Cyclopropylmethyl)[(2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]amino}-*N*-(pyridin-3-ylmethyl)benzamide (23k). ^{1}H NMR (DMSO-d_6) δ: 8.79 (dd, J = 5, 5 Hz, 1H), 8.70 (s, 1H), 8.65 (d, J = 5 Hz, 1H), 8.13 (d, J = 1 Hz, 1H), 7.92 (d, J = 1 Hz, 1H), 7.79−7.59 (m, 5H), 7.55 (d, J = 8 Hz, 1H), 6.75 (d, J = 8 Hz, 2H), 4.84 (s, 2H), 4.52 (d, J = 5 Hz, 2H), 3.50 (s, 3H), 3.46 (d, J = 6 Hz, 2 Hz), 1.19−1.08 (m, 1H), 0.49−0.42 (m, 2H), 0.31−0.25 (m, 2H). HRMS [M + H] calculated for $C_{28}H_{29}N_5O_2$ 468.2394, found 468.2393.

4-{(Cyclopropylcarbonyl)[(2,3-dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl]amino}-*N*-(pyridin-3-ylmethyl)benzamide (23l). ^{1}H NMR (DMSO-d_6) δ: 9.22 (dd, J = 5, 5 Hz, 1H), 8.75 (s, 1H), 8.68 (d, J = 4 Hz, 1H), 8.18 (d, J = 7 Hz, 7.91−7.84 (m, 3H), 7.76 (dd, J = 7, 5, 5 Hz, 1H), 7.61 (dd, J = 8, 1, 1 Hz, 1H), 5.09 (s, 1H), 4.57 (d, J = 6, 2H), 3.50 (s, J = 2H), 2.57 (s, 3H), 1.41 (bs, 1H), 0.97−0.88 (m, 2H), 0.75−0.66 (m, 2H). HRMS [M + H] calculated for $C_{28}H_{27}N_5O_3$ 482.218 67, found 482.218 67.

4-{[(2,3-Dimethyl-4-oxo-3,4-dihydroquinazolin-6-yl)methyl](3-methylbut-2-enoyl)amino}-*N*-(pyridin-3-ylmethyl)benzamide (23m). ^{1}H NMR (DMSO-d_6) δ: 9.24 (dd, J = 5, 5 Hz, 1H), 8.97−8.56 (m, 2H), 8.26 (d, J = 7 Hz, 1H), 7.90 (d, J = 2 Hz, 1H), 7.87−7.79 (m, 3H), 7.65 (dd, J = 8, 2, 2 Hz, 1H), 7.53 (d, J = 8 Hz, 2H), 5.53 (s, 1H), 5.11 (s, 2H), 4.58 (d, J = 5 Hz, 2H), 3.51 (s, 3H), 2.58 (s, 3H), 2.08 (s, 3H), 1.68 (s, 3H). HRMS [M + H] calculated for $C_{29}H_{29}N_5O_3$ 496.234 32, found 496.234 56.

4-(Prop-2-ynylamino)-*N*-(3-pyridylmethyl)benzamide (24). Propargyl bromide is added to ethyl 4-aminobenzoate (1.2 equiv) and 2,6-lutidine in DMF. The solution is heated at 70 °C overnight, concentrated, and purified by MPLC (0−30% EtOAc/hex). The resulting solid is dissolved in ethanol, and 2 M NaOH (2 equiv) is added. The mixture is heated at reflux for 2.5 h. The ethanol is removed under vacuum, ethyl acetate is added, and the organic layer is washed with 10% HCl, dried with Na_2SO_4, and concentrated. DCM is added followed by 3-aminomethylpyridine, DIC (1.2 equiv), HOBt (1.2 equiv), and DIEA (3 equiv). The solution is stirred at room temperature overnight, concentrated, and purified by MPLC (0−20% MeOH/DCM).

4-[{[7-Chloro-2-(hydroxymethyl)-3-methyl-4-oxo-3,4-dihydroquinazolin-6-yl]methyl}(prop-2-yn-1-yl)amino]-*N*-(pyridin-3-ylmethyl)benzamide (26). ^{1}H NMR (DMSO-d_6) δ: 8.90 (t, J = 5.7 Hz, 1H), 8.71 (s, 1H), 8.65 (d, J = 2.2 Hz, J = 1H), 8.13 (d, J = 4.3 Hz, 1H), 7.91 (s, 1H), 7.83 (s, 1H), 7.80−7.69 (m, 3H), 6.80 (d, J = 4.3 Hz, 2H), 4.79 (s, 2H), 4.58 (s, 2H), 4.54 (d, J = 2.7 Hz, 2H), 4.39 (s, 2H), 3.50 (s, 3H), 3.24 (d, J = 2.0 Hz, 1H). HRMS [M + H] calculated for $C_{27}H_{24}ClN_5O_3$ 502.1648, found 502.1687.

7-Chloro-3-methyl-4-oxoquinazoline-6-carboxylic Acid (28). Quinazolinone **13** is dissolved in water/pyridine (2:1; 0.3 M) and heated to 100 °C. Potassium permanganate (7 equiv) is added in portions at 100 °C, and the solution is heated at reflux for 5 h. and filtered over Celite. The filtrate is dilluted with EtOAc, washed with 10% HCl, dried with Na_2SO_4, and concentrated. The acid is used crude.

7-Chloro-3-methyl-4-oxo-*N*-[4-(3-pyridylmethylcarbamoyl)-phenyl]quinazoline-6-carboxamide (29). TEPP (1.2 equiv) is added to acid **28** and 4-amino-*N*-(3-pyridylmethyl)benzamide in toluene and the solution heated at reflux overnight. The solution was concentrated and purified by RP-HPLC. ^{1}H NMR (DMSO-d_6) δ: 10.92 (s, 1H), 9.12 (t, J = 5.9 Hz, 1H), 8.68 (d, J = 1.6 Hz, 1H), 8.59 (dd, J = 4.6, 1.6 Hz, 1H), 8.52 (s, 1H), 8.30 (s, 1H), 8.00

(d, J = 7.7 Hz, 1H), 7.92 (d, J = 9.0 Hz, 2H), 7.91 (s, 1H), 7.82 (d, J = 8.6 Hz, 2H), 7.60 (dd, J = 8.5, 5.0 Hz, 1H), 4.56 (d, J = 5.9 Hz, 2H), 3.53 (s, 3H). HRMS [M + H] calculated for $C_{23}H_{18}ClN_5O_3$ 448.117 09, found 448.123 38.

6-Amino-7-chloro-3-methylquinazolin-4-one (30). DPPA and Et_3N are added to acid **28** in *t*-BuOH, and the solution is heated at reflux overnight. Ethyl acetate is added and washed with saturated $NaHCO_3$, dried with Na_2SO_4, and concentrated. DCM followed by TFA is added and the solution stirred at room temperature for 90 min and then concentrated. Amine **30** is used crude.

4-[[(7-Chloro-3-methyl-4-oxo-quinazolin-6-yl)amino]methyl]-*N*-(3-pyridylmethyl)benzamide (31). TFA (2 equiv) is added to amine **30** and 4-formylbenzoic acid in isopropyl acetate and the solution stirred at room temperature for 10 min. $NaBH(OAc)_3$ (1.5 equiv) is added and the mixture heated at reflux overnight, concentrated, and purified by MPLC (0−20% MeOH/DCM). The resulting acid is added to 3-aminomethylpyridine (1.2 equiv), DIC (1.2 equiv), HOBt (1.2 equiv) in DMF. DIEA (3 equiv) is added and the solution stirred overnight at room temperature, concentrated, and purified by RP-HPLC. ^{1}H NMR (DMSO-d_6) δ: 9.15 (t, J = 5.5 Hz, 1H), 8.75 (d, J = 1.5 Hz, 1H), 8.68 (dd, J = 4.3, 1.5 Hz, 1H), 8.20 (d, J = 7.4 Hz, 1H), 8.12 (s, 1H), 7.84 (d, J = 8.2 Hz, 2H), 7.77 (dd, J = 7.4, 5.5 Hz, 1H), 7.68 (s, 1H), 7.45 (d, J = 8.6 Hz, 2H), 6.98 (s, 1H), 6.86 (bs, 1H), 4.58 (m, 4H), 3.38 (s, 3H). HRMS [M + H] calculated for $C_{23}H_{20}ClN_5O_2$ 434.137 83, found 434.138 57.

2-Amino-6-methyl-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-4-one (32)/5-Methylfuro[2,3-*d*]pyrimidine-2,4-diamine[14b] (33). Chloracetone is added to 2,6-diamino-3,4-dihydropyrim-4-one in DMF and the solution heated at 50 °C overnight, concentrated, and purified by MPLC (0−20% MeOH/DCM). Fraction 1 contains **32**. Fraction 2 contains **33**.

4-[(2-Amino-6-methyl-4-oxo-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-5-yl)sulfanyl]benzoic Acid (34). Heterocycle **32**, 4-mercaptobenzoic acid (1.75 equiv), is heated at reflux in 2:1 ethanol/water for 5 min. Iodine (1.75 equiv) is added and the solution heated at reflux for 3 h, cooled, and filtered. A solution of 10% $Na_2S_2O_3$ is added to the filtrate and the mixture stirred at room temperature overnight, filtered, concentrated, and carried on crude.

4-[(2-Amino-6-methyl-4-oxo-3,7-dihydropyrrolo[2,3-*d*]pyrimidin-5-yl)sulfanyl]-*N*-(3-pyridylmethyl)benzamide (35). 3-Aminomethylpyridine (1.2 equiv), DIC (1.2 equiv), HOBt (1.2 equiv) are added to **34** followed by DIEA (3 equiv). The solution is stirred at room temperature overnight, concentrated, and purified by RP-HPLC. ^{1}H NMR (DMSO-d_6) δ: 9.12 (t, J = 5.8 Hz, 1H), 8.61 (d, J = 2.0 Hz, 1H), 8.54 (dd, J = 4.6, 2.0 Hz, 1H), 7.88 (d, J = 7.0 Hz, 1H), 7.82 (d, J = 9.0 Hz, 2H), 7.54−7.48 (m, 1H), 7.20 (d, J = 8.6 Hz, 2H), 4.51 (d, J = 5.5 Hz, 2H), 2.33 (s, 3H). HRMS [M + H] calculated for $C_{20}H_{18}N_6O_2S$ 407.128 47, found 407.125 27.

5-Methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine-2,4-diamine (36). Et_3N is added to malononitrile and hydroxyacetone in MeOH and the solution stirred at room temperature overnight and concentrated. The resulting solid is dissolved in ethanol and added to a premixed solution of guanidine hydrochloride (1.5 equiv) and NaOEt (1.5 equiv) in EtOH. The reaction mixture is heated at reflux overnight, concentrated, and purified by MPLC (0−20% MeOH/DCM).

4-[(2,4-Diamino-6-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)-sulfanyl]-*N*-(pyridin-3-ylmethyl)benzamide (37). Addition of 4-mercaptobenzoic acid and subsequent amide coupling proceed as described for **35**−**36**. ^{1}H NMR (DMSO-d_6) δ: 9.10 (t, J = 5.9 Hz, 1H), 8.59 (s, 1H), 8.53 (d, J = 4.0 Hz, 1H), 7.81 (d, J = 8.6 Hz, 2H), 7.48 (t, J = 5.0 Hz, 1H), 7.30 (bs, 1H), 7.12 (d, J = 8.6 Hz, 2H), 4.50 (d, J = 6.3 Hz, 2H), 2.34 (s, 3H). HRMS [M + H] calculated for $C_{20}H_{19}N_7OS$ 406.144 46, found 406.133 72.

4-[(2,4-Diamino-6-methyl-1*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)-sulfanyl]-*N*-[2-(pyridin-3-yl)ethyl]benzamide (38). Addition of 4-mercaptobenzoic acid and subsequent amide coupling proceed as described for **35**−**36**. ^{1}H NMR (DMSO-d_6) δ: 8.60−8.51 (m, 3H), 7.94 (d, J = 7.4 Hz, 1H), 7.71 (d, J = 8.6 Hz, 2H), 7.56 (dd, J = 7.8, 5.0 Hz, 1H), 7.31 (bs, 2H), 7.09 (d, J = 8.6 Hz, 2H), 3.52 (q, J = 7.0 Hz, 2H), 2.92 (t, J = 7.0 Hz, 2H), 2.35 (s, 3H). HRMS [M + H] calculated for $C_{21}H_{21}N_7OS$ 420.160 11, found 420.155 15.

4-[(3-Chloro-2-oxopropyl)prop-2-ynylamino]-*N*-(3-pyridylmethyl)benzamide (39). *tert*-Butyl bromoacetate is added to **24** and 2,6-lutidine in DMF, and the solution is heated at 70 °C overnight, concentrated, and purified by MPLC (0−20% MeOH/DCM). The resulting oil is dissolved in DCM, TFA is added, and the mixture is stirred for 2 h and then concentrated. The acid is then dissolved in DCM. Oxalyl chloride (2 equiv) is added followed by 0.5 mL of DMF. THF is added after 5 min to aid in solubility. The solution is stirred for 1 h and then concentrated. THF is followed by CH_2N_2 (2 M in hexanes, 2 equiv). The mixture is stirred at room temperature for 4 h, then cooled to 0 °C and HCl (g) is bubbled through the solution for 10 min. The resulting solid is filtered, dried under vacuum, and carried on crude.

4-[(2,4-Diaminofuro[2,3-*d*]pyrimidin-5-yl)methylprop-2-ynylamino]-*N*-(3-pyridylmethyl)benzamide (40). 2,6-Diamino-3,4-dihydropyrim-4-one is added to chloride **39** in DMF and the solution heated at 70 °C overnight and 150 °C for an additional 4 h. The solution is filtered, washed with a small amount of MeOH, and the solid is purified by RP-HPLC. ^{1}H NMR (DMSO-d_6) δ: 8.94 (s, 1H), 8.93−8.81 (m, 2H), 8.46 (d, J = 8.2 Hz, 1H), 8.08 (dd, J = 8.2, 6.3 Hz, 1H), 7.71 (d, J = 8.6 Hz, 2H), 6.67 (d, J = 9.0 Hz, 2H), 4.59 (d, J = 5.8 Hz, 2H), 4.35 (s, 2H), 3.94 (d, J = 2.4 Hz, 2H), 3.12 (t, J = 2.4 Hz, 1H). HRMS [M + H] calculated for $C_{23}H_{21}N_7O_2$ 427.175 67.

4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoic Acid (41). Et_3N (20 mol %) is added to ethyl 4-(3-oxopropyl)-benzoate, paraformaldehyde, and 3-ethylthiazoline bromide in EtOH. The solution is heated at reflux overnight, concentrated, and purified by MPLC (0−100% EtOAc/hex). The resulting hydroxyketone is added to malononitrile and Et_3N in MeOH and the solution stirred at room temperature overnight and filtered. The solid is added to a premixed (5 min) solution of guanidine hydrochloride (1.5 equiv) and KOEt (1.5 equiv) in EtOH and the mixture heated at reflux overnight. The mixture was concentrated and the resulting acid carried on crude.

4-[2-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]-*N*-(3-pyridylmethyl)benzamide (42). DMF, 3-aminomethylpyridine (1.2 equiv), DIC (1.2 equiv), HOBt (1.2 equiv), and DIEA (3 equiv) are added to acid **41**. The solution is stirred at room temperature overnight, concentrated, and purified by RP-HPLC. ^{1}H NMR (DMSO-d_6) δ: 11.48 (s, 1H), 9.07 (t, J = 5.8 Hz, 1H), 8.58 (d, J = 1.6 Hz, 1H), 8.50 (dd, J = 4.7, 1.7 Hz, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.79 (bs, 1H), 7.44 (dd, J = 7.8, 5.1 Hz, 1H), 7.35 (d, J = 8.2 Hz, 2H), 6.59 (bs, 2H), 4.51 (d, J = 5.9 Hz, 2H), 3.06−2.98 (m, 2H), 2.96−2.90 (m, 2H). HRMS [M + H] calculated for $C_{21}H_{21}N_7O$ 388.188 03, found 388.178 32.

2-Methyl-5,6,7,8-tetrahydro-3*H*-pyrido[4,3-*d*]pyrimidin-4-one (43). To a round bottomed flask methyl 4-oxopiperidine-3-carboxylate (1.0 equiv) and acetamidine HCl (1.1 equiv) were added with stirring in EtOH at room temperature. While the mixture was being stirred, Na (2.1 equiv) was dissolved in another flask containing EtOH. Upon dissolution, the above mixture was added to the mixture containing the acetamidine mixture, and the mixture was heated at 100 °C overnight. The reaction mixture was removed from heat, and upon cooling the mixture was filtered to remove solids, then concentrated to yield 2-methyl-5,6,7,8-tetrahydro-3*H*-pyrido[4,3-*d*]pyrimidin-4-one as a cream colored solid.

2-Methyl-4-oxo-*N*-[4-(3-pyridylmethylcarbamoyl)phenyl]-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidine-6-carboxamide (44). To a round bottomed flask **43** (1.0 equiv) was added with stirring in DCM at room temperature. To this solution ethyl 4-isocyanatobenzoate (1.1 equiv) was slowly added. As the reaction proceeded, solid crashed out of solution. After being stirred for 1.5 h, the

reaction mixture was filtered and the isolated precipitate was rinsed with DCM and dried to yield ethyl 4-[(2-methyl-4-oxo-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidine-6-carbonyl)amino]benzoate as a white solid.

To a round-bottomed flask ethyl 4-[(2-methyl-4-oxo-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidine-6-carbonyl)amino]benzoate (1.0 equiv) was added with stirring in 1 N LiOH (5−10 equiv) at room temperature overnight. After the reaction was determined to be complete, the mixture was neutralized with HCl, at which point a white solid crashed out of solution. The solid was filtered and collected to yield 4-[(2-methyl-4-oxo-3,5,7,8-tetrahydropyrido-[4,3-*d*]pyrimidine-6-carbonyl)amino]benzoic acid.

To a medium vial 4-[(2-methyl-4-oxo-3,5,7,8-tetrahydropyrido-[4,3-*d*]pyrimidine-6-carbonyl)amino]benzoic acid (1.0 equiv), EDC (1.5 equiv), and HOBt (1.5 equiv) were added with stirring in dry DMF. 3-Aminomethylpyridine (1.1 equiv) was added followed by TEA (3.0 equiv). The mixture was stirred at room temperature overnight and purified via flash chromatography using 0−20% DCM/MeOH. ^{1}H NMR (DMSO-d_6) δ: 9.10−9.02 (m, 2H), 8.81 (s, 1H), 8.73 (d, J = 4.0 Hz, 1H), 8.29 (d, J = 4.4 Hz, 1H), 7.88−7.83 (m, 1H), 7.80 (d, J = 5.6 Hz, 2H), 7.60 (d, J = 5.6 Hz, 2H), 4.59 (d, J = 2.8 Hz, 2H), 4.31 (s, 2H), 3.70 (t, J = 5.7 Hz, 2H), 2.64 (t, J = 5.8 Hz, 2H), 2.31 (s, 3H). HRMS [M + H] calculated for $C_{22}H_{22}N_6O_3$ 419.1834, found 419.1839.

4-(2-Methyl-4-oxo-3,5,7,8-tetrahydropyrido[4,3-*d*]pyrimidine-6-carbonyl)-*N*-(3-pyridylmethyl)benzamide (45). To a medium vial **43** (1.0 equiv), EDC (1.5 equiv), and HOBt (1.5 equiv) were added with stirring in dry DMF. 4-(3-Pyridylmethylcarbamoyl)benzoic acid (1.1 equiv) was added followed by TEA (3.0 equiv). The mixture was stirred at room temperature overnight and purified via flash chromatography using 0−20% DCM/MeOH. ^{1}H NMR (DMSO-d_6) δ: 9.35 (t, J = 6.5 Hz, 1H), 8.81 (s, 1H), 8.72 (d, J = 2.9 Hz, 1H), 8.27 (t, J = 7.0 Hz, 1H), 7.97 (d, J = 4.4 Hz, 2H), 7.82 (t, J = 6.9 Hz, 1H), 7.58 (d, J = 4.4 Hz, 2H), 4.63 (d, J = 3.0 Hz, 2H), 4.41 (bs, 1H), 4.15 (bs, 1H), 3.91−8.83 (m, 1H), 3.56−3.44 (m, 1H), 2.69−2.59 (m, 2H), 2.28 (d, J = 5.2 Hz, 3H). HRMS [M + H] calculated for $C_{22}H_{21}N_5O_3$ 404.1724, found 404.1720.

Biological Evaluation. The coupled-enzyme Nampt enzymatic assay, PARP cellular Nampt activity assay, and HCT116 72 h cytotoxicity assay in the presence and absence of nicotinic acid were done as previously described.[4]

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Supporting Information Available: Experimental procedure for molecular modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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