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Development of Small-Molecule Modulators of Nucleotide Metabolism Enzymes

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Development of Small-Molecule Modulators of Nucleotide Metabolism Enzymes

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy in Chemistry

by

Juno Simone Van Valkenburgh

2019

ABSTRACT OF THE DISSERTATION

Development of Small-Molecule Modulators of Nucleotide Metabolism Enzymes

by

Juno Simone Van Valkenburgh
Doctor of Philosophy in Chemistry
University of California, Los Angeles, 2019
Professor Michael E. Jung, Chair

In chapter 1, strategies toward the asymmetric synthesis of a deoxycytidine kinase (dCK) inhibitor are presented. Small molecule dCK inhibitors are potential cancer therapeutics: in combination with inhibition of the *de novo* deoxyribonucleotide triphosphate biosynthetic pathway, they have been shown to be effective against acute lymphoblastic leukemia in animal models. Our group previously identified a series of chiral dCK inhibitors, of which only the *R*-enantiomer is responsible for kinase inhibition; we thus sought an asymmetric synthesis of these molecules. We pursued a synthetic route in which an S_N2 substitution at the chiral center occurs early in the synthesis, to avoid racemization due to a competing S_N1 mechanism, which has been observed in a previous asymmetric synthesis from our group. We utilized (–)-ethyl L-lactate as a starting material, as it contains the required chiral carbon skeleton as well as readily-transformable functional groups. Our initial efforts using a Takai-Utimoto olefination as a key step were unsuccessful. Further strategies were hindered by the reactivity of the 4,6-diaminopyrimidine

moiety introduced through the early substitution reaction, and ultimately a successful route was not reached.

In chapter 2, the development of α-*N*-heterocyclic carboxaldehyde thiosemicarbazone (HCT) compounds as anti-proliferative agents is described. HCTs have long been known to have anti-cancer properties, due to various mechanisms which generally involve chelation to a redox active metal. One notable HCT which is an iron chelator is Triapine (3-AP), which is the most promising currently-available ribonucleotide reductase inhibitor. Despite currently being in Phase II clinical trials, 3-AP has poor pharmacokinetic properties, so we developed a series of 3-AP analogs which retain the pyridine scaffold of 3-AP but have modifications on the terminal amine of the thiosemicarbazone. None had significantly improved properties over 3-AP, however. HCTs with an isoquinoline scaffold have also previously been developed as ribonucleotide reductase inhibitors but were not pursued clinically due to poor drug-like properties. We synthesized a series of isoquinoline-based HCTs, several of which synergize strongly with physiologically relevant levels of Cu(II) supplementation. The lead compound 6-fluoroisoquinoline-1-carboxaldehyde *N*,*N*-dimethylthiosemicarbazone (**2-79)** exhibits nanomolar IC_{90} values in the presence of copper, giving it potential as a cancer therapeutic.

The dissertation of Juno Simone Van Valkenburgh is approved.

Craig A. Merlic

Caius G. Radu

Michael E. Jung, Committee Chair

University of California, Los Angeles

2019

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The synthesis of (*R*)-DI-82 presented in Scheme 1-1 and the structure of compound **2-51** were proposed by Professor Michael Jung.

Chapter 2 is adapted from:

Sun, D.; Poddar, S.; Pan, R.D.; Van Valkenburgh, J.; Rosser, E.W.; Abt, E.R.; Lok, V.; Capri, J.; Hernandez, S.P.; Song, J.; Li, J.; Vergnes, L.; Cabebe, A.; Armstrong, W.; Plamthottam, S.; Steele, S.; Osto, C.; Stuparu, A.; Le, T.M.; Damoiseaux, R.; Czernin, J.; Satyamurthy, N.; Jung, M.E. and Radu, C.G. Evaluation of potent isoquinoline-based thiosemicarbazone antiproliferatives against solid tumor models. In preparation.

Biological assays were performed by members in the lab of Prof. Caius Radu. Soumya Poddar determined IC_{50} and IC_{90} values and performed proliferation and viability assays and FACS analysis. Compounds **2-49**, **2-51**, **2-52**, **2-62**, **2-63**, **2-69 – 71**, **2-73 – 76**, **2-78**, and **2-79** were synthesized by Daniel Sun. ICP-MS analysis was performed by Shane Que Hee at the UCLA ICP-MS core facility.

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Sun, D.; Poddar, S.; Pan, R.D.; **Van Valkenburgh, J.;** Rosser, E.W.; Abt, E.R.; Lok, V.; Capri, J.; Hernandez, S.P.; Song, J.; Li, J.; Vergnes, L.; Cabebe, A.; Armstrong, W.; Plamthottam, S.; Steele, D.; Osto, C.; Stuparu, A.; Le, T.M.; Damoiseaux, R.; Czernin, J.; Satyamurthy, N.; Jung, M.E.; Radu, C.G. Evaluation of potent isoquinoline-based thiosemicarbazone antiproliferatives against solid tumor models. In preparation.

Plamthottam, S.; Sun D.; **Van Valkenburgh, J.;** Valenzuela, J.; Ruehle, B.; Steele, D.; Poddar, S.; Hernandez, S.; Satyamurthy, N.; Radu, C.; Zink, J. Development of iron binding assays to explore structure activity relationships of 3-AP analogs. Submitted for review.

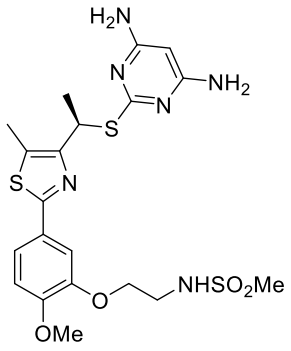
Kim, W.; Le, T. M.; Wei, L.; Poddar, S.; Bazzy, J.; Wang, X.; Uong, N. T.; Abt, E. R.; Capri, J. R.; Austin, W. R.; **Van Valkenburgh, J.;** Steele, D.; Gipson, R. M.; Slavik, R.; Cabebe, A. E.; Taechariyakul, T.; Yaghoubi, S. S.; Lee, J. T.; Sadeghi, S.; Lavie, A.; Faull, K. F.; Witte, O. N.; Donahue, T. R.; Phelps, M. E.; Herschman, H. R.; Herrmann, K.; Czernin, J.; Radu, C. G. [^{18}F]CFA as a clinically translatable probe for PET imaging of deoxycytidine kinase activity. *Proceedings of the National Academy of Sciences* **2016**, *113*(15), 4027–4032.

CHAPTER 1

Progress Toward the Asymmetric Synthesis of a Deoxycytidine Kinase Inhibitor

INTRODUCTION

Two major pathways, the *de novo* pathway (DNP) and the nucleoside salvage pathway (NSP), are responsible for nucleotide biosynthesis in mammalian cells. Both pathways are used to maintain deoxyribonucleotide triphosphate (dNTP) pools for DNA replication and repair. In the NSP, extracellular deoxycytidine, deoxyadenosine, and deoxyguanosine are phosphorylated by deoxycytidine kinase (dCK) into the corresponding monophosphates that are then further phosphorylated to form dNTPs.[1] In addition to its function in nucleotide metabolism, dCK is also activated in response to DNA damage and is required for regulating the G2/M checkpoint in cancer cells.[2] Furthermore, dCK is instrumental in activating several clinically important nucleoside analog prodrugs of anticancer and antiviral agents via phosphorylation.[3] Additionally, our lab has shown that dCK is required for hematopoiesis in lymphoid and erythroid progenitors.[4,5] We have also shown that in acute lymphoblastic leukemia cells, partial inhibition of the DNP in combination with a small molecule dCK inhibitor caused cells to experience replication stress while normal hematopoietic cells were not affected.[6] The biological activity of dCK and its potential as a therapeutic target led our group to become interested in small-molecule inhibitors of the enzyme.



(*R*)-DI-82, **1-1**

Figure 1-1. (*R*)-DI-82 **(1-1)**.

High throughput screening of a large library of molecules identified hit inhibitor compounds which were optimized through structure-activity relationship studies, resulting in lead compounds that inhibited uptake of tritiated deoxycytidine in CCRF-CEM cells with nanomolar potency.[7] Further optimization to improve metabolic stability and potency led to identification of (*R*)-DI-82 (**1-1**, Figure 1-1).[8]

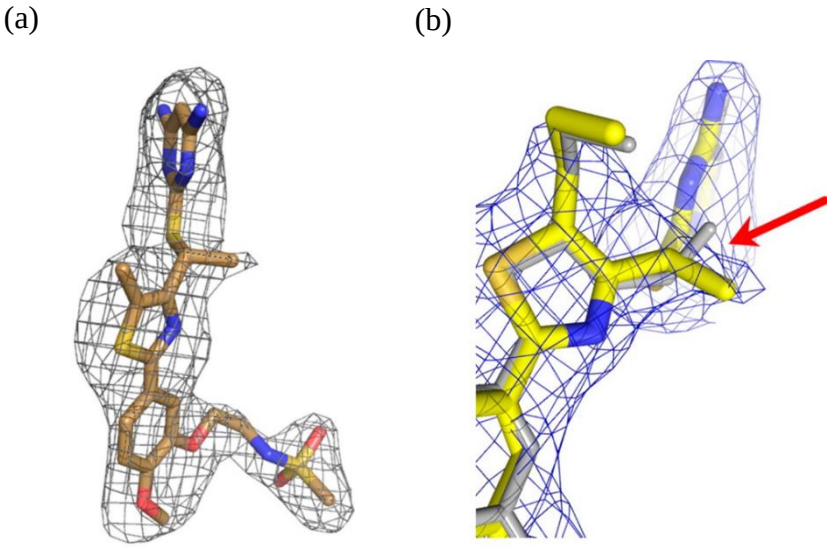


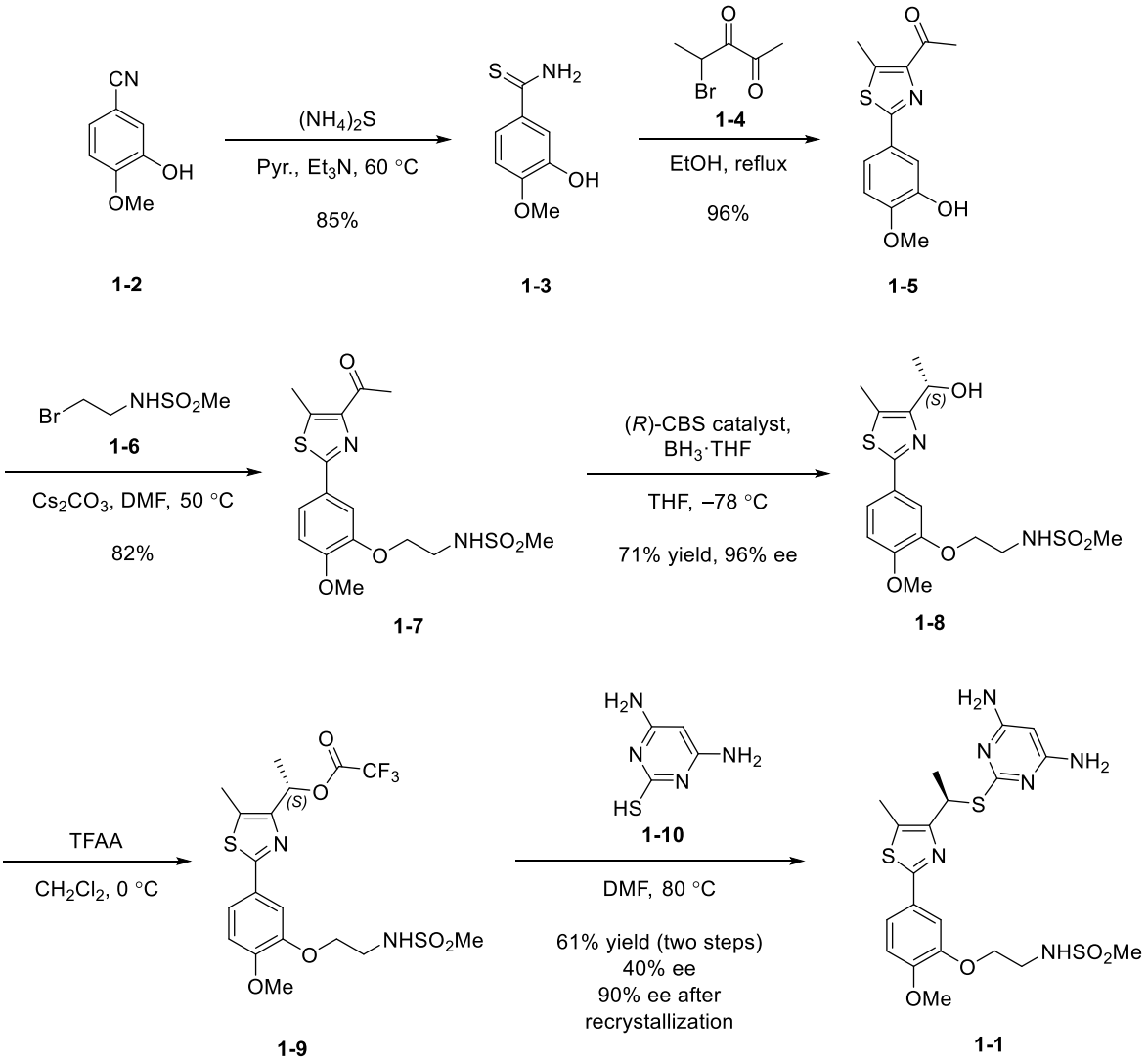
Figure 1-2. 1-1 and its enantiomer show differential binding with dCK. (a) dCK crystallized with **1-1**. (b) Earlier-generation analog of **1-1** bound with dCK (yellow) with its *S*-isomer (***ent*-1-1**) modelled in the binding pocket (gray). The linker methyl groups are indicated by a red arrow.[8]

Table 1-1. *in vitro* (IC_{50}^{app} and K_i^{app}) and cell (IC_{50}) properties of **1-1** and its enantiomer.[8]

	Steady state kinetics		CEM cells
Compound	IC_{50}^{app} (nM)	K_i^{app} (nM)	IC_{50} (nM)
(*S*)-DI-82 (***ent*-1-1**)	605 ± 108	585 ± 104	94.0 ± 14.4
(*R*)-DI-82 (**1-1**)	27.8 ± 3.5	9.2 ± 1.1	3.7 ± 0.8

1-1 and analogs bind to the dCK active site, with the pyrimidine ring occupying a very similar position to the pyrimidine of dC, the natural substrate.[9] The chiral center of the inhibitor plays an important role in the binding affinity as shown in Figure 1-2; the crystal structure of **1-1** bound to dCK shows a good fit in the binding pocket in (a), with the methyl group on the chiral center fitting very well, and a computational model of an analog of (*S*)-DI-82 (***ent*-1-1**) showing a very poor fit in (b). Table 1-1 shows that the **1-1** has a much higher binding affinity *in vitro* and is

Scheme 1-1. Our group's previous synthesis of (*R*)-DI-82 (**1-1**).

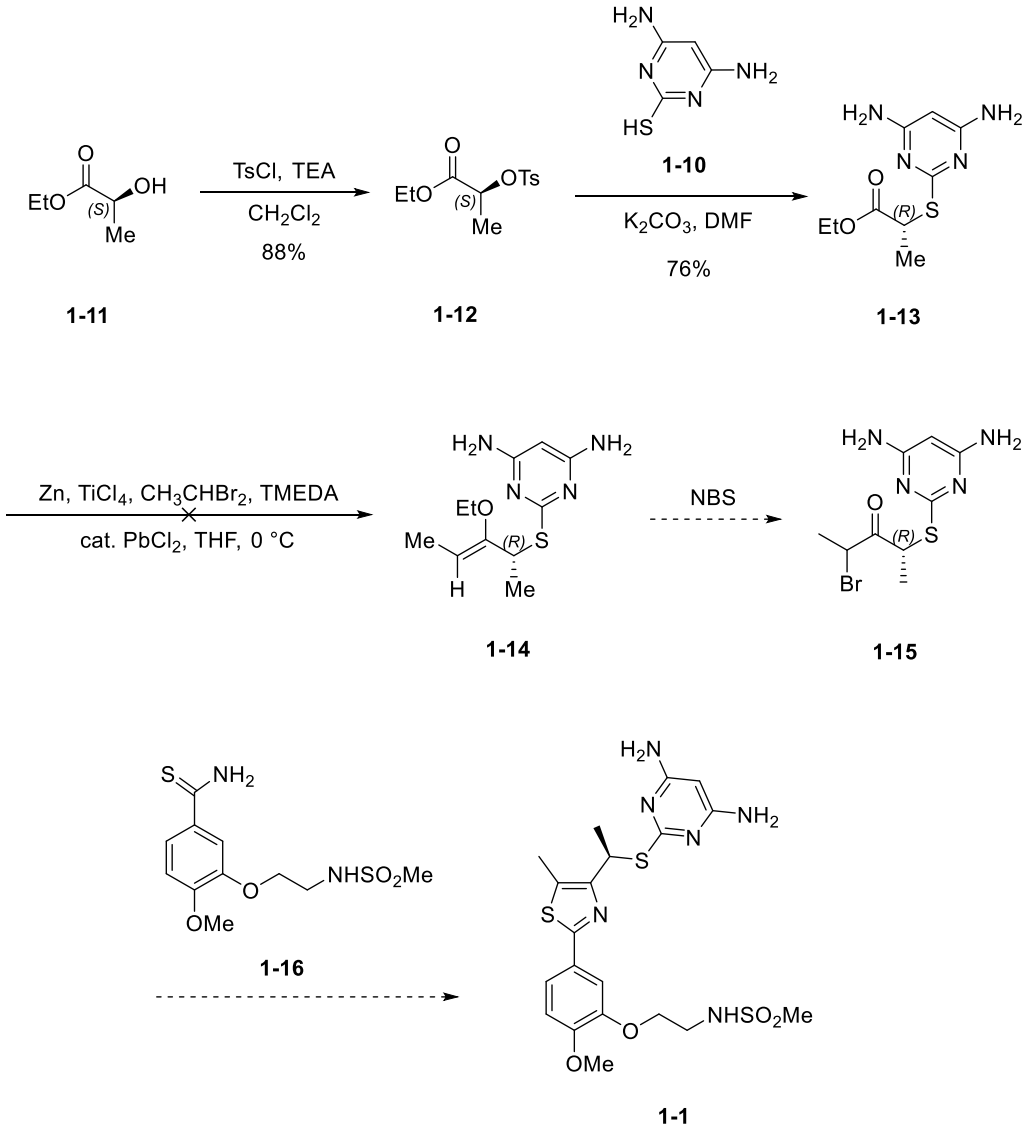


more active in cells.[8] Because of the difference in potency between the enantiomers, an asymmetric synthesis is necessary to obtain the enantiopure molecule.

Our group has previously reported an asymmetric synthesis of **1-1**.[8] The commercially available 3-hydroxy-4-methoxybenzonitrile (**1-2**) was treated with aqueous ammonium sulfide under basic conditions to form the thioamide **1-3**, which was then condensed with 4-bromo-2,3-pentanedione (**1-4**) to form the thiazole core of **1-5**. The phenol ring was alkylated with *N*-(2-bromoethyl)methanesulfonamide (**1-6**) to afford the ketone **1-7**. Chiral reduction of the ketone to the alcohol **1-8** was achieved with Corey-Bakshi-Shibata (CBS) reduction with an enantiomeric excess of 96%. Functionalizing the alcohol to the corresponding sulfonates using mesic or tosic anhydride led to elimination to the alkene, likely due to the adjacent thiazole stabilizing the carbocation intermediate. The trifluoroacetate **1-9** was formed by treating alcohol **1-8** with trifluoroacetic anhydride without a significant amount of racemization or elimination. Reacting **1-9** with 4,6-diaminopyrimidine-2-thiol (**1-10**) gave **1-1** in 61% yield over two steps with 40% enantiomeric excess. The partial racemization suggests that the reaction occurs partially via an S_N2 mechanism, and partially by an S_N1 pathway, presumably due to the carbocation intermediate stabilized by the thiazole, similar to how the elimination reaction of derivatives of **1-8** was favored. Chiral resolution through recrystallization in methanol and acetone increased the enantiomeric excess to over 90%, but dramatically decreased the yield.

In this work we explore possible synthetic routes toward **1-1** that would be practical to implement on large enough scale to allow for assaying *in vivo* biological activity and eventual drug production. We sought a route that utilizes the chiral pool to introduce stereocenters, and that reduces racemization eliminating the need for chiral resolution.

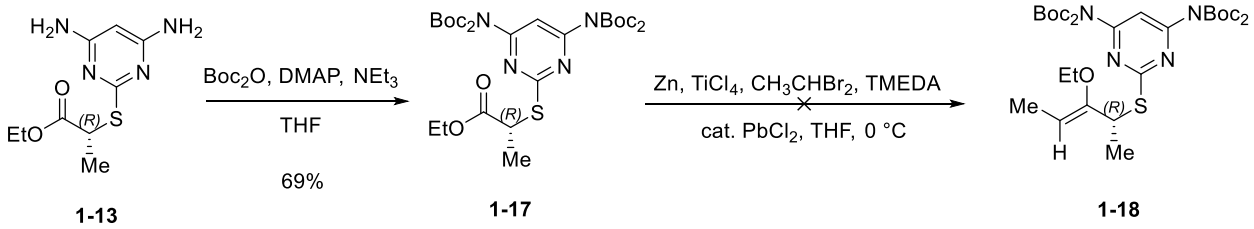
RESULTS AND DISCUSSION



Scheme 1-2. Proposed synthesis of **1-1**.

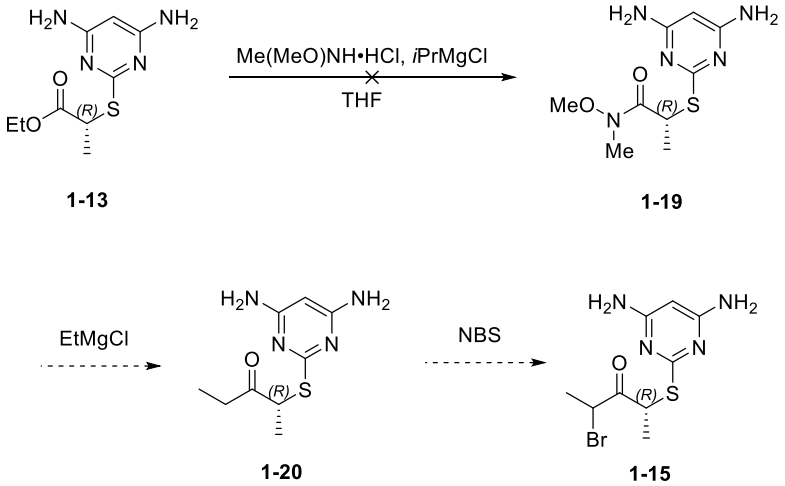
Our planned synthetic route, proposed by Professor Michael Jung, is shown in Scheme 1-2. We began with (–)-ethyl L-lactate (**1-11**), which is very inexpensive and contains a stereocenter which, upon substitution, provides the correct configuration and the methyl substituent for the (*R*)-stereocenter of **1-1**. The substitution reaction to introduce the mercaptopyrimidine moiety to the chiral center to form **1-13** occurs before formation of the thiazole ring, in contrast to our previous

synthetic route. Because there would only be a carbonyl to stabilize the carbocation intermediate and not a benzylic-like system, the S_N2 pathway should be favored over the S_N1 pathway and therefore lead to a lesser degree of racemization than in the previous synthesis. The reaction of the mesylate of (–)-ethyl L-lactate and thiophenol is known to proceed with enantiomeric excess of up to 99%, and we expected our system to behave similarly.[10] To install the carbon atoms that would become the 5-position of the thiazole and its methyl substituent, a Takai-Utimoto olefination could be used, which is known for simple esters, and the protocol has been developed as an ester-alkene metathesis method.[11,12,13] Treating ester **1-13** with zinc, titanium tetrachloride, catalytic lead chloride, and 1,1-dibromoethane would furnish the enol ether **1-14**, which could then be brominated with *N*-bromosuccinimide to alpha-bromoketone **1-15**. Formation of the thiazole core could then proceed by heating **1-15** and thioamide **1-16** together to give **1-1**.[8] The forward synthesis began smoothly with tosylation of **1-11** to **1-12** in 88% yield. The diaminopyrimidine moiety was introduced via treatment of **1-12** and **1-10** with potassium carbonate to give **1-13**. The Takai-Utimoto reaction, however, was not successful and **1-14** was not synthesized, as the reaction only returned starting material.



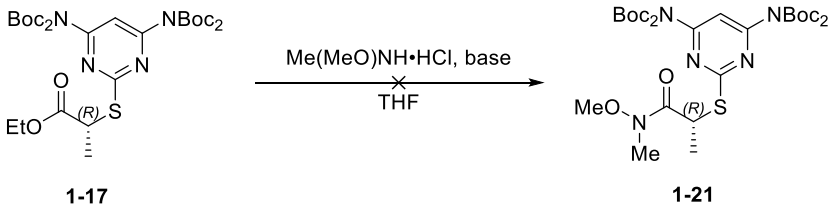
Scheme 1-3. Attempt to perform Takai-Utimoto olefination using protected substrate.

We hypothesized that the reactivity of the amines could be contributing to the failure of the olefination reaction, so **1-13** was doubly Boc-protected on both amines to give **1-17**, which was subjected to the Takai-Utimoto conditions (Scheme 1-3). Unfortunately the enol ether **1-18** was not formed; instead decomposition and some recovered starting material resulted.



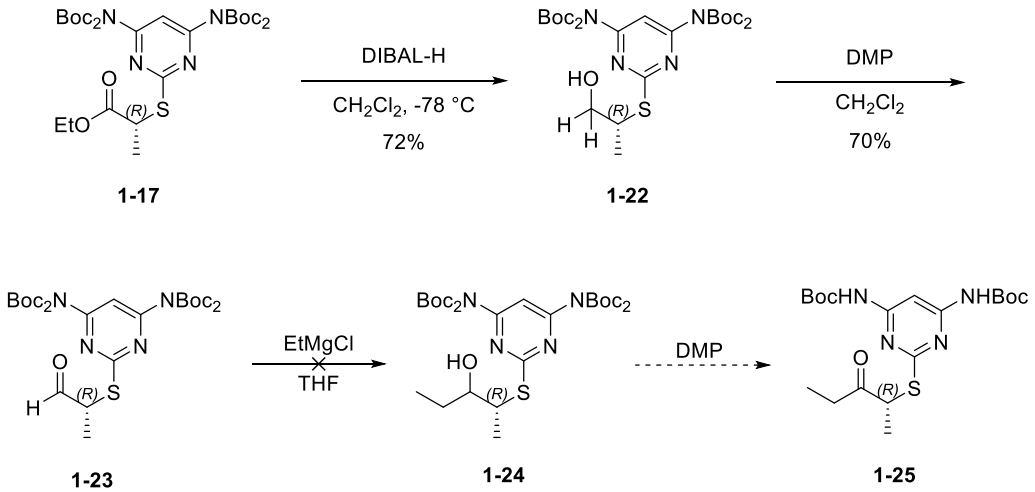
Scheme 1-4. Attempt to form bromoketone **1-15** using Weinreb amide intermediate **1-19**.

Table 1-2. Attempt to form Weinreb amide **1-21**.



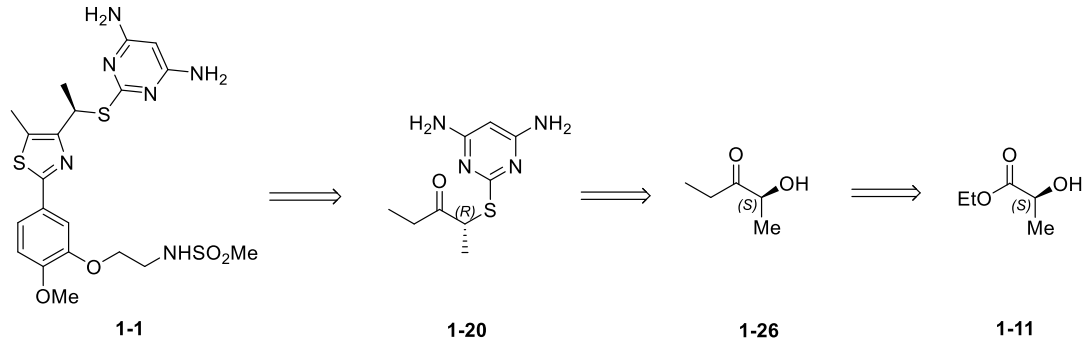
Entry	Base	Result
1	*i*PrMgCl	Complex mixture
2	EtMgCl	Complex mixture
3	2-MesMgBr	N.R.
4	LiHMDS	Complex mixture

Subsequently, we turned our attention to an alternative proposed forward synthesis that circumvents the failed olefination reaction (Scheme 1-4). Ester intermediate **1-13** would be transformed into bromoketone **1-15** similarly to the original planned synthesis. In the new approach, **1-13** would be converted into Weinreb amide **1-19**. Grignard addition to form ketone **1-20** would be followed by bromination to reach **1-15**. Treatment of **1-13** with *N*,*O*-dimethylhydroxylamine hydrochloride and isopropylmagnesium chloride gave no reaction, however. Again reasoning that this could be due to the amines on the pyrimidine ring, we attempted the Weinreb amide formation with Boc-protected **1-17**, but again were unsuccessful**.** Table 1-2 shows the results of treatment of **1-17** with *N*,*O*-dimethylhydroxylamine hydrochloride and various bases to attempt to form the Weinreb amide **1-21**. In entries 1 and 2, Grignard reagents gave complex mixtures of products, possibly due to the lability of the Boc protecting groups to nucleophiles, so we turned to non-nucleophilic bases as in entries 3 and 4. 2-Mesitylmagnesium bromide gave no reaction, while lithium bis(trimethylsilyl)amide also resulted in a complex mixture.



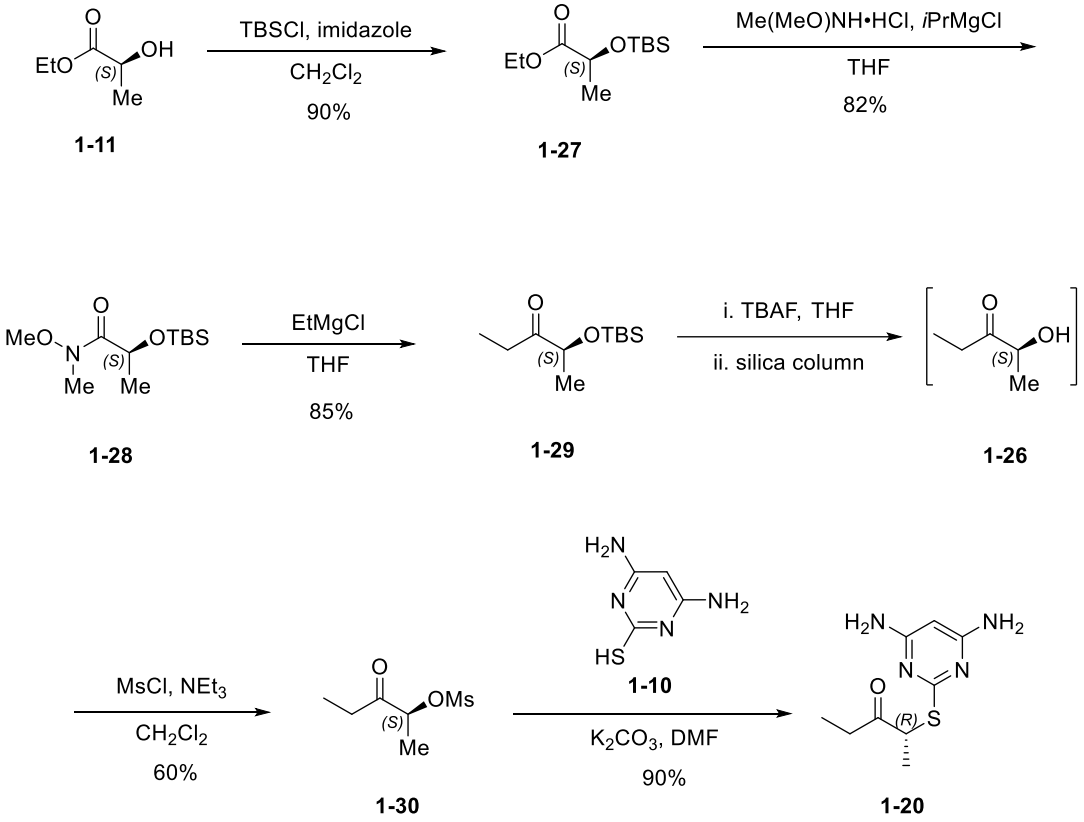
Scheme 1-5. Alternative synthesis of ketone **1-25**.

Another possible method to form the ketone **1-25,** analogous to **1-20** with Boc-protected amines, is shown in Scheme 1-5. The ester **1-17** could be reduced to the aldehyde **1-23** with diisobutylaluminum hydride. Addition to the carbonyl could be accomplished with ethylmagnesium chloride to form secondary alcohol **1-24,** which could undergo oxidation with Dess-Martin periodinane to ketone **1-25.** Reduction of **1-17** did not proceed to the aldehyde **1-23** directly, but to the primary alcohol **1-22**, which was then oxidized with Dess-Martin periodinane to give the aldehyde **1-23**. Addition of ethyl Grignard to the aldehyde failed, however, and **1-24** was not formed, giving a mixture of starting material and decomposition products.



Scheme 1-6. Revised retrosynthetic analysis of **1-1**.

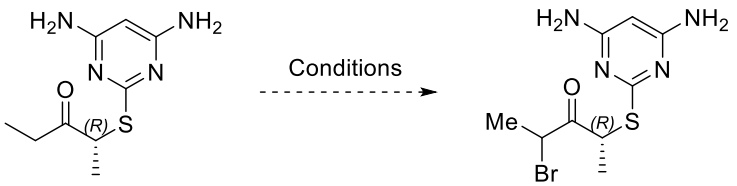
Because of this result, we decided to revisit our retrosynthetic analysis to make **1-1**. As shown in Scheme 1-6, we decided to retain ketone **1-20** as a key intermediate, but form it instead via substitution on **1-26**, in which the ketone backbone is already formed from (–)-ethyl L-lactate (**1-11**). This strategy avoids many reactions in the presence of the highly reactive diaminopyrimidine moiety, while still performing the substitution reaction at the chiral center before formation of the thiazole to avoid racemization.



Scheme 1-7. Forward synthesis of **1-20**.

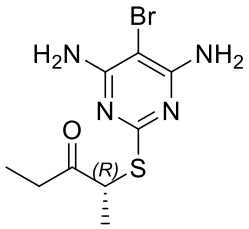
The revised forward synthesis is shown in Scheme 1-7. The alcohol of **1-11** was protected with *tert*-butyldimethylsilyl chloride to give **1-27**, which was transformed into Weinreb amide **1-28** in very good yield. Ketone **1-29** was formed smoothly upon treatment with ethylmagnesium chloride. The product of the silyl deprotection reaction **1-26** was not isolated as it proved to be very volatile. Instead, **1-29** was treated with tetrabutylammonium fluoride, and the reaction mixture was directly loaded onto a silica plug and eluted with dichloromethane to remove the byproducts of the reaction. The eluted solvent containing the product was then partially concentrated, and the mixture was directly subjected to mesyl chloride and triethylamine to give mesylate **1-30** in 60% yield. The substitution reaction with **1-10** in basic conditions gave **1-20** in excellent yield.

Table 1-3. Bromination of **1-20**.



1-20 **1-31**

Entry	Conditions	Result
1	Br_2, MeOH	**32**
2	Br_2, LiHMDS, THF	**32**
3	Br_2, NaHMDS, THF	**32**
4	Br_2, 2-MesMgBr, THF	**32**
5	Br_2, LDA, THF	**32**
6	NBS, LiHMDS, THF	N.R.
7	NBS, NH_4OAc, Et_2O	**32**
8	$PhMe_3NBr_3$, THF	**32**
9	$CuBr_2$, EtOAc	Complex mixture



1-32

Figure 1-3. Side product **1-32**.

With ketone **1-20** in hand, we attempted to brominate the methylene carbon alpha to the carbonyl to form **1-31**. Table 1-3 shows conditions attempted for the bromination of **1-20**. Simply reacting **1-20** with liquid bromine as in entry 1 gave bromination not of the methylene carbon, but at the 5-position of the pyrimidine ring (**1-32**, Figure 1-3).[14] Reactions were monitored by ^{1}HNMR with formation of the side product indicated by disappearance of the aromatic proton at 5.27 ppm. Addition of strong bases such as lithium bis(trimethylsilyl)amide, sodium bis(trimethylsilyl)amide, 2-mesitylmagnesium bromide, and lithium diisopropylamide to promote deprotonation alpha to the ketone gave the same result (entries 2-5). In contrast, treatment with *N*-bromosuccinimide and lithium bis(trimethylsilyl)amide gave no reaction, while treating **1-20** with *N*-bromosuccinimide and ammonium acetate again gave **1-32**. Treatment with trimethylphenylammonium tribromide also resulted in **1-32**.[15] Subjecting **1-20** to copper(II)bromide in ethyl acetate resulted in a complex mixture of products with no monobrominated product visible by LCMS.

We hypothesized that decreasing the electron density on the pyrimidine ring would make bromination on the ring less favorable, and so we decided to transform the amines into electron-withdrawing carbamates. Attempts at Boc-protection of the amines on the pyrimidine led to a mixture of di-, tri-, and tetracarbamates that were inseparable by column chromatography. Further attempts at protection were not pursued due to time constraints.

CONCLUSION

Strategies toward the asymmetric synthesis of a deoxycytidine kinase inhibitor have been explored. Utilizing (–)-ethyl L-lactate as a starting material provided a chiral skeleton for the stereocenter and the surrounding moieties of the lead compound. Our efforts were stymied by the reactivity of the 4,6-diaminopyrimidine ring linked to the stereocenter which had to be introduced early in the synthesis to avoid racemization, but which had a propensity toward side reactions during transformations on other parts of the molecule, and we were ultimately unsuccessful.

EXPERIMENTAL

General

All chemicals, reagents and solvents were obtained from commercial sources and were used without further purification. Unless otherwise noted, reactions were carried out in oven-dried glassware under an atmosphere of argon using commercially available anhydrous solvents. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride under an argon atmosphere. Solvents used for extractions and chromatography were not anhydrous. Analytical TLC was carried out on precoated silica gel plates (Merck silica gel 60, F254) and visualized with UV light or permanganate stain. Column chromatography was performed with silica (Fisher, 230-400 mesh). ^{1}H NMR and ^{13}C NMR, spectra were measured in $CDCl_3$ or DMSO-d_6 on Bruker AV spectrometers at 300 or 500 MHz. Chemical shifts were reported in parts per million (δ) relative to residual solvent signals. The signals observed were described as follows: s (singlet), d (doublet), t (triplet), q (quartet), dq (doublet of quartets), qd (quartet of doublets), m (multiplet), br s (broad singlet). Mass spectra were obtained on a Waters LCT Premier with ACQUITY UPLC mass spectrometer under electrospray ionization (ESI) or Thermo Fisher Scientific Exactive Plus with direct analysis in real time (DART) ionization.

Ethyl (*S*)-2-(tosyloxy)propanoate (1-12). To a stirred solution of (–)-ethyl L-lactate (**1-11**) (5.93 g, 50.1 mmol) and p-toluenesulfonyl chloride (TsCl, 11.35 g, 59.5 mmol) in dichloromethane (25 ml) was added dropwise triethylamine (10.4 mL, 75.1 mmol). The mixture was stirred for 5 h, washed with water (3 x 15 ml) and dried over Na_2SO_4. The solvent was removed *in vacuo* and the

reaction was purified by flash column chromatography on silica gel (10% ethyl acetate/hexanes) to yield the tosylate **1-12** (11.98 g, 87.8% yield) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ

7.78 (d, *J* = 8.3 Hz, 2H)

7.31 (d, *J* = 8.0 Hz, 2H)

4.88 (q, *J* = 6.9 Hz, 1H)

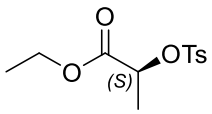
4.08 (q, *J* = 7.1 Hz, 1H)

4.07 (q, *J* = 7.2 Hz, 1H)

2.41 (s, 3H)

1.47 (d, *J* = 6.9 Hz, 3H)

1.17 (t, *J* = 7.2 Hz, 3H).



1-12

^{13}C NMR (500 MHz, $CDCl_3$) δ 169.03, 145.10, 133.35, 129.80, 127.98, 74.18, 61.79, 21.62, 18.37, 13.91.

HRMS-ESI: *m/z* calcd for $C_{12}H_{16}O_5S$ $(M+H)^+$ 273.07912, found 273.08061.

Ethyl (*R*)-2-((4,6-diaminopyrimidin-2-yl)thio)propanoate (1-13). 4,6-Diaminopyrimidine-2-thiol (**1-10**) (0.548 g, 3.85 mmol) was dissolved in DMF (5 mL), freshly distilled from lithium aluminum hydride, and potassium carbonate (0.540 g, 3.91 mmol) was added followed by the tosylate **1-12** (0.995 g, 3.65 mmol). The reaction was stirred for 3 h at 23 °C. The solid was filtered off and washed with THF. The solvent was removed and the resulting oil was purified by flash

column chromatography on silica gel (15% ethyl acetate/hexanes) to yield the sulfide **1-13** (0.811 g, 91.6% yield) as a pale yellow powder.

^{1}H NMR (500 MHz, DMSO-d_6) δ

6.12 (br s, 4H)

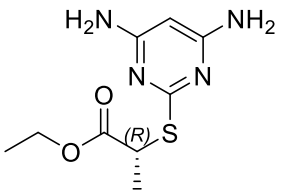
5.14 (s, 1H)

4.47 (q, J = 7.2 Hz, 1H)

4.10 (q, J = 7.1 Hz, 2H)

1.44 (d, J = 7.2 Hz, 3H)

1.18 (t, J = 7.1 Hz, 3H).



1-13

^{13}C NMR (125 MHz, DMSO-d_6) δ 172.91, 167.26, 163.93, 79.64, 61.17, 41.20, 18.85, 14.45.

HRMS-ESI: *m/z* calcd for $C_9H_{15}N_4O_2S$ $(M+H)^+$ 243.09102, found 243.09121.

Ethyl (*R*)-2-((4,6-bis(bis(*tert*-butoxycarbonyl)amino)pyrimidin-2-yl)thio)propanoate (1-17). The diamine **1-13** (0.05 g, 0.21 mmol) was dissolved in THF (2.5 mL) at 23 °C and triethylamine (0.086 mL, 0.62 mmol) was added. The reaction was cooled to 0 °C and 4-dimethylaminopyridine (DMAP, 3.4 mg, 0.021 mmol) and di-*tert*-butyl dicarbonate (Boc_2O, 0.095 mL, 0.413 mmol) were added. The reaction was stirred overnight. The solvent was removed and the residue diluted with ethyl acetate (3 mL) and washed with saturated aqueous NH_4Cl (5 mL) and brine (5 mL). The organic fraction was dried over $MgSO_4$, filtered, and concentrated. The resulting oil was purified

by flash column chromatography on silica gel (8% ethyl acetate/hexanes) to give the tetracarbamate **1-17** (0.093 g, 69% yield) as a colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ

7.62 (s, 1H)

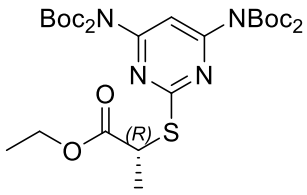
4.51 (q, *J* = 7.2 Hz, 1H)

4.18 (q, *J* = 7.2 Hz, 2H)

1.57 (d, *J* = 7.2 Hz, 3H)

1.53 (s, 36 H)

1.25 (t, *J* = 7.2 Hz, 3H).



1-17

^{13}C NMR (125 MHz, $CDCl_3$) δ 172.15, 168.50, 159.15, 149.95, 99.93, 84.34, 61.51, 42.16, 27.76, 18.39, 14.04.

HRMS-ESI: *m/z* calcd for $C_{29}H_{47}N_4O_{10}S$ $(M+H)^+$ 643.3013, found 643.3007.

di-*tert*-Butyl(2-((1-hydroxypropan-2-yl)thio)pyrimidine-4,6-diyl)(*R*)-bis((*tert*-butoxycarbonyl)carbamate) (1-22). To a solution of the ester **1-17** (0.020 g, 0.031 mmol) in dichloromethane (3 mL) at –41˚C was added DIBAL-H (0.062 mL, 0.062 mmol, 1 M in THF) dropwise over 10 minutes. The mixture was stirred for 2 h then quenched with water and filtered through Celite. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated. The resulting solid was purified by flash column chromatography on silica gel (gradient, 20-25% ethyl acetate/hexanes) to give the alcohol **1-22** as a white solid (0.012 g, 64% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

7.61 (s, 1H)

3.96 – 3.87 (m, 1H)

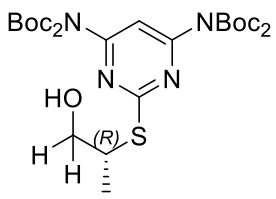
3.78 – 3.70 (m, 1H)

3.69 – 3.61 (m, 1H)

2.25 (s, 1H)

1.53 (s, 36H)

1.35 (d, *J* = 7.3 Hz, 3H).



1-22

^{13}C NMR (125 MHz, $CDCl_3$) 169.70, 159.01, 150.05, 99.46, 84.44, 67.24, 42.32, 27.77, 16.67.

DART-MS: *m/z* calcd for $C_{27}H_{45}N_4O_9S$ $(M+H)^+$ 601.29018, found 601.30017.

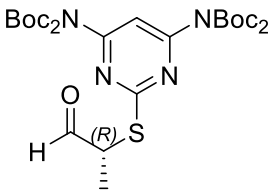
(*R*)-2-((4,6-Bis(bis(*tert*-butoxycarbonyl)amino)pyrimidin-2-yl)thio)propanal (1-23). To a cooled (0 ˚C) solution of alcohol **1-22** (0.018 g, 0.030 mmol) in dichloromethane (0.5 mL) was added Dess-Martin periodinane (DMP, 0.015 g, 0.036 mmol). The mixture was warmed to room temperature and stirred for 6 h. The reaction was quenched with saturated aqueous $NaHCO_3$ (0.5 mL) and saturated aqueous $Na_2S_2O_3$ (0.5 mL) and the mixture was stirred for 10 min. The phases were separated and the aqueous phase was washed with dichloromethane (2 x 1 mL). The combined organic layers were washed with brine (2 mL), dried over Na_2SO_4, and concentrated. The resulting solid was dissolved in minimal dichloromethane and purified by flash column chromatography on silica gel (10% ethyl acetate/hexanes) to give the aldehyde **1-23** as a colorless oil (0.017 g, 94% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

9.58 (d, *J* = 0.6 Hz, 1H)

7.67 (s, 1H)

4.41 (qd, *J* = 7.3, 0.7 Hz, 1H)



1-23

1.53 (s, 36H)

1.43 (d, *J* = 7.3 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 197.22, 167.74, 159.26, 149.93, 100.19, 84.55, 47.08, 27.76, 12.77.

DART-MS: *m/z* calcd for $C_{27}H_{43}N_4O_9S$ $(M+H)^+$ 599.27453, found 599.27390.

Ethyl (S)-2-((*tert*-butyldimethylsilyl)oxy)propanoate (1-27). To a solution of (–)-ethyl L-lactate (**1-11**) (1.5 g, 12.69 mmol) in dichloromethane (50 mL) was added imidazole (1.739 g, 2.54 mmol). The solution was then cooled to 0 °C and *tert*-butyldimethylsilyl chloride (TBSCl, 2.488 g, 16.51 mmol) was added one granule at a time, upon which a white precipitate formed. The solution was allowed to warm to room temperature and stir for 4 h. The reaction was filtered and the solid washed with dichloromethane. The filtrate was collected and washed sequentially with HCl (8 mL, 1 M in H_2O), saturated aqueous $NaHCO_3$ (8 mL), and brine (8 mL). The resulting organic phase was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product wad purified by flash column chromatography on silica gel (8% ethyl acetate/hexanes) to yield the ester **1-27** as colorless oil (2.66 g, 90% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

4.26 (q, *J* = 6.8 Hz, 1H)

4.19 – 4.06 (m, 2H)

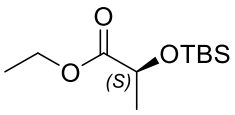
1.35 (d, *J* = 6.8 Hz, 3H)

1.23 (t, *J* = 7.2 Hz, 3H)

0.86 (s, 9H)

0.06 (s, 3H)

0.03 (s, 3H).



1-27

^{13}C NMR (125 MHz, $CDCl_3$) δ 174.03, 68.42, 60.66, 25.69, 21.26, 18.28, 14.16, -4.98, -5.32.

HRMS-ESI: *m/z* calcd for $C_{11}H_{25}O_3Si$ $(M+H)^+$ 233.15675, found 233.15460.

(*S*)-2-((*tert*-Butyldimethylsilyl)oxy)-*N*-methoxy-*N*-methylpropanamide (1-28). To a solution of the ester **1-27** (0.200 g, 0.861 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (0.176 g, 1.807 mmol) in THF (5 mL) was added isopropylmagnesium chloride solution (1.81 mL, 3.61 mmol, 2 M in THF) dropwise over 20 min at –10 °C. The reaction was allowed to stir at 0 °C for 50 min. A solution of saturated aqueous NH_4Cl (6 mL) was added and the phases were separated. The aqueous phase was extracted with diethyl ether (4 x 4 mL) then dichloromethane (2 x 4 mL) and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The product was purified by column chromatography on silica gel (18% ethyl acetate/hexanes) to yield the amide **1-28** as a colorless oil (0.174 g, 82% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

4.64 (q, *J* = 6.9 Hz, 1H)

3.66 (s, 3H)

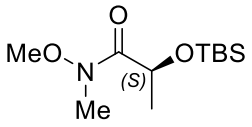
3.17 (s, 3H)

1.32 (d, *J* = 6.6 Hz, 3H)

0.86 (s, 9H)

0.06 (s, 3H)

0.04 (s, 3H).



1-28

^{13}C NMR (125 MHz, $CDCl_3$) δ 174.87, 66.41, 61.16, 32.52, 25.78, 20.87, 18.29, -4.74, -5.03.

DART-MS: *m/z* calcd for $C_{11}H_{26}NO_3Si$ $(M+H)^+$ 248.41690, found 248.16656.

(*S*)-2-((*tert*-Butyldimethylsilyl)oxy)pentan-3-one (1-29). To a solution of the amide **1-28** (0.14 g, 0.606 mmol) at 0 °C in THF (3 mL) was added ethylmagnesium chloride solution (0.65 mL, 1.94 mmol, 2.7 M in THF). The mixture was allowed to warm to room temperature and stir for 40 min. A solution of saturated aqueous NH_4Cl (4 mL) was added and the aqueous phase was extracted with diethyl ether (5 mL) and dichloromethane (2 x 5 mL) and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel (2.5% ethyl acetate/hexanes) to yield the ketone **1-29** as a colorless oil (0.103 g, 85% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

4.14 (q, *J* = 6.8 Hz, 1H)

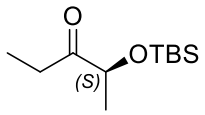
2.74 – 2.45 (m, 2H)

1.26 (d, *J* = 6.8 Hz, 3H)

1.02 (t, *J* = 7.3 Hz, 3H)

0.90 (s, 9H)

0.06 (s, 6H).



1-29

DART-MS: *m/z* calcd for $C_{11}H_{25}O_2Si$ $(M+H)^+$ 217.16183, found 217.16106.

(*S*)-3-Oxopentan-2-yl methanesulfonate (1-30). To a round-bottom flask containing **1-29** (0.900 g, 4.16 mmol) was added tetrabutyl ammonium fluoride solution (TBAF, 8.32 mL, 8.32 mmol, 1 M in THF) and the mixture was stirred for 3 h. The mixture was loaded directly onto a silica gel column and eluted with dichloromethane. The eluted solvent containing the desired compound was concentrated from approximately 500 mL to 200 mL and used directly in the next reaction. To the solution was added mesyl chloride (0.386 mL, 4.99 mmol) followed by dropwise addition of triethylamine (0.869 mL, 6.24 mmol). The mixture was stirred for 15 h then concentrated *in vacuo*. The residue was dissolved in dichloromethane (50 mL) and washed with water (3 x 50 mL) and brine (50 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (25% ethyl acetate/hexanes) to yield the mesylate **1-30** as a yellow oil (0.462 g, 62% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

5.07 (q, *J* = 7.0 Hz, 1H)

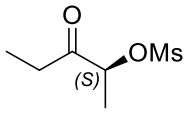
3.12 (s, 3H)

2.60 (q, *J* = 7.2 Hz, 1H)

2.59 (q, *J* = 7.2 Hz, 1H)

1.54 (d, *J* = 7.0 Hz, 3H)

1.09 (t, *J* = 7.2 Hz, 3H).



1-30

^{13}C NMR (125 MHz, $CDCl_3$) δ 207.02, 80.11, 39.02, 31.53, 17.68, 7.12.

DART-MS: *m/z* calcd for $C_6H_{13}O_4S$ $(M+H)^+$ 181.05291, found 181.05341.

(*R*)-2-((4,6-Diaminopyrimidin-2-yl)thio)pentan-3-one (1-31). To a solution of 4,6-diaminopyrimidine-2-thiol (**1-10**) (0.304 g, 2.14 mmol) in acetonitrile (15 mL) was added K_2CO_3 (0.295 g, 2.14 mmol) then the mesylate **1-30** (0.350 g, 1.94 mmol). The solution was heated at 55 °C and stirred for 48 h. The mixture was filtered and water (10 mL) was added, forming a single phase which was washed with dichloromethane (10 mL). The organic phase was washed with water (10 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (gradient, 60-75% ethyl acetate/hexanes) to yield the sulfide **1-31** as a white solid (0.397 g, 92% yield).

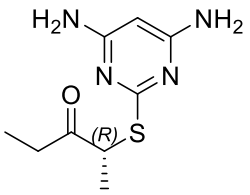
^{1}H NMR (500 MHz, $CDCl_3$) δ

5.27 (s, 1H)

4.61 (br s, 4H)

4.43 (q, *J* = 7.3 Hz, 1H)

2.79 (dq, *J* = 17.8, 7.3 Hz, 1H)



1-31

2.59 (dq, *J* = 17.8, 7.3 Hz, 1H)

1.47 (d, *J* = 7.3 Hz, 3H)

1.06 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 169.11, 163.00, 80.72, 47.62, 32.44, 16.31, 8.24. One downfield carbon was not observed.

DART-MS: *m/z* calcd for $C_6H_{13}O_4S$ $(M+H)^+$ 227.09611, found 227.09497.

(*R*)-2-((4,6-Diamino-5-bromopyrimidin-2-yl)thio)pentan-3-one (1-32). To a solution of the ketone **1-31** (0.025 g, 0.110 mmol) in methanol (5 mL) at –45 ˚C was added Br_2 (5.7 µL, 0.110 mmol). The reaction was stirred for 30 minutes and warmed to room temperature then quenched with saturated aqueous $NaHCO_3$ (0.5 mL). After 5 min, the reaction was diluted with water (5 mL) then extracted with ethyl acetate (4 x 10 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (10 mL), water (10 mL), and brine (10 mL), and dried over Na_2SO_4 to give the sulfide **1-32** as a yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ

5.14 (br s, 4H)

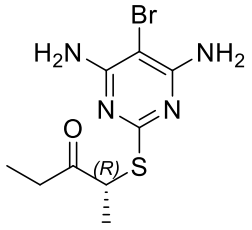
4.34 (q, *J* = 7.3 Hz, 1H)

2.77 (dq, *J* = 17.9, 7.3 Hz, 1H)

2.58 (dq, *J* = 17.9, 7.3 Hz, 1H)

1.46 (d, *J* = 7.3 Hz, 3H)

1.05 (t, *J* = 7.3 Hz, 3H).



1-32

DART-MS: *m/z* calcd for $C_{27}H_{45}N_4O_9S$ $(M+H)^+$ 305.00662, found 305.00531.

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CHAPTER 2

Development of α-*N*-heterocyclic Carboxaldehyde Thiosemicarbazones as Cancer Therapeutics

INTRODUCTION

α-N-heterocyclic carboxaldehyde thiosemicarbazones (HCTs) are a diverse class of molecules that have been investigated for their therapeutic potential for decades. They have been shown to have antiproliferative activity in tumors, viruses, bacteria, and fungi.[1-7] One of the mechanisms of action that some HCTs are thought to function by is inhibition of ribonucleotide reductase (RNR).[8] RNR catalyzes the reduction of ribonucleotides to their corresponding deoxyribonucleotides, building blocks for DNA synthesis and repair.[9] The enzyme is composed of dimers of two different subunits, called R1 and R2. Each R1 subunit contains an active site where substrate binding occurs, and allosteric sites that control overall activity and substrate specificity. The R2 subunit contains a diferric iron center and a tyrosyl radical that are necessary for enzyme activity.[10] RNR is extremely important for rapidly proliferating cells such as cancer cells, and is in fact upregulated in many types of cancer.[11]

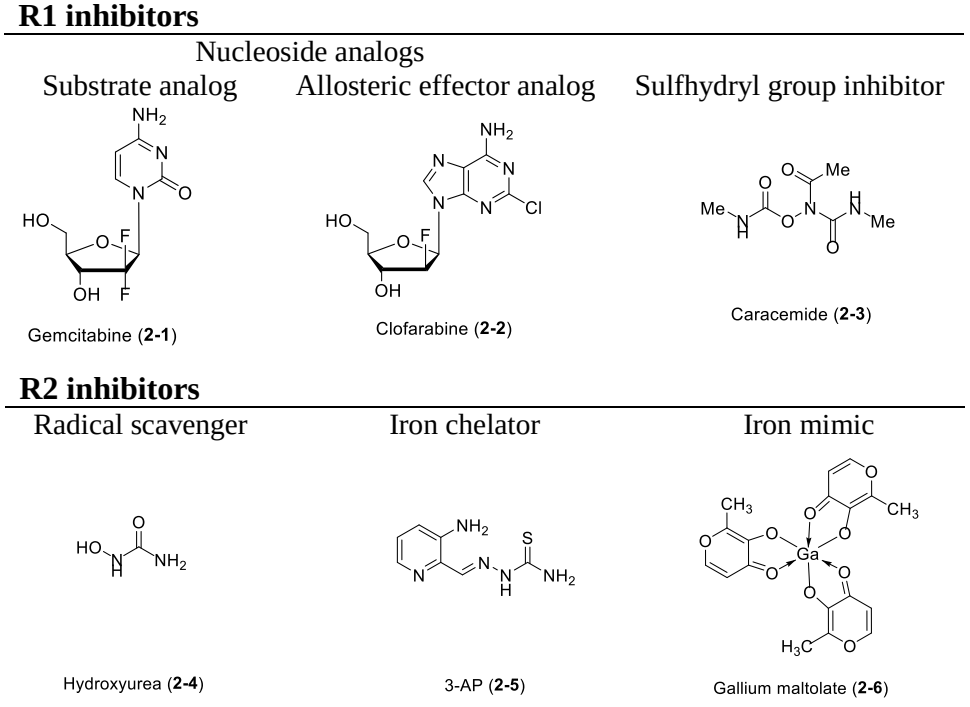


Figure 2-1. Clinically relevant RNR inhibitors.

There are many known inhibitors of both subunits of RNR. Figure 2-1 shows some inhibitors that are being investigated in the clinic. Among the R1 subunit inhibitors are nucleoside analogs such as gemcitabine (**2-1**) which mimic the substrates, and clofarabine (**2-2**) which upon phosphorylation mimics ATP, an allosteric regulator of RNR.[12] Carecemide (**2-3**) is a covalent inhibitor that has not progressed beyond phase II clinical trials because of severe toxicity.[13] Among the R2 subunit inhibitors, hydroxyurea (**2-4**) scavenges the tyrosyl radical so the radical cannot propagate to the active site.[12] The HCT R2 subunit inhibitor 3-AP, also called Triapine (**2-5**) functions by quenching the tyrosyl radical.[13] 3-AP has been shown to be a potent RNR inhibitor, and has undergone multiple clinical trials for the treatment of various cancers, but has not progressed beyond phase II.[12-20] Another inhibitor of the R2 subunit is gallium maltolate (**2-6**), a bioavailable form of gallium, which functions by replacing the iron in the R2 subunit so it can no longer initiate the radical cascade required for activity.

The number and variety of inhibitors of RNR that have been developed is testament to its desirability as a target for cancer therapeutics. We became interested in RNR as a target in the *de novo* nucleotide biosynthesis pathway to complement our small-molecule inhibitors of the nucleotide salvage pathway in a combination therapy.[21] Out of the many RNR inhibitors available, we focused our efforts on 3-AP (**2-5**) because of its HCT structure. Since HCTs are well-studied and showed strong anti-proliferative activity, we thought this compound was a promising starting point for new drug development.

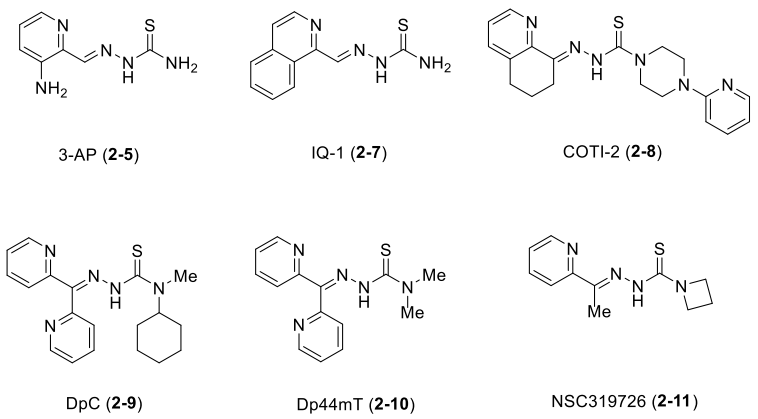


Figure 2-2. Clinically relevant HCT compounds.

Isoquinoline-based HCTs were also thought to be RNR inhibitors, and were studied extensively but were essentially abandoned after the development of 3-AP.[4] These compounds, including IQ-1 (**2-7**) were the subject of early interest due to their efficacy, particularly in terms of 50-day survival rates of tumor-bearing mice (Figure 2-2).[3,5] Despite the extensive studies of these compounds, there is still chemical space around this scaffold for development of new compounds.

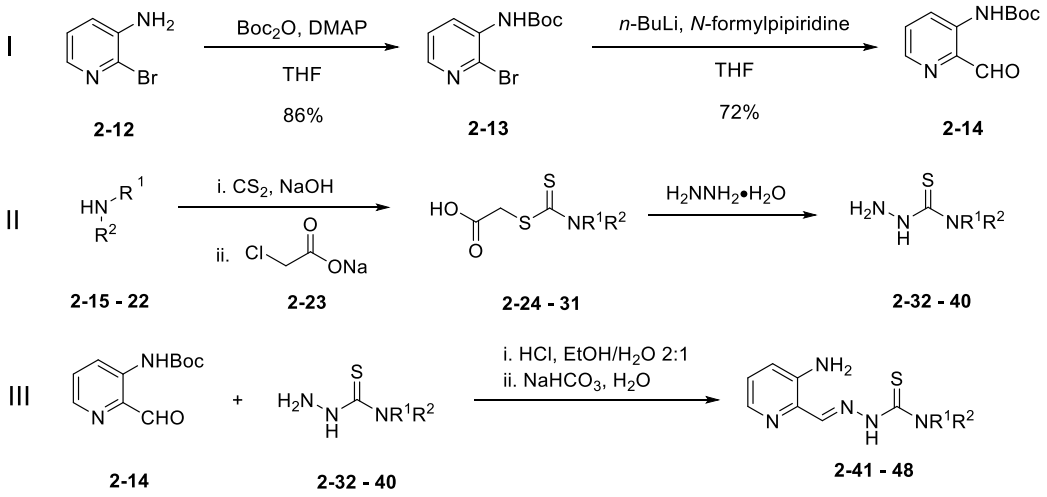
HCTs have been shown to have many different mechanisms of action beyond RNR inhibition. For instance, COTI-2 (**2-8**) restores mutant p53 activity, and DpC (**2-9**) increases expression and phosphorylation of growth and metathesis suppressors such as NDRG1 (Figure 2-2).[22,23] These compounds have been investigated in the clinic, but they nor any other HCT compounds have advanced beyond phase II clinical trials.[19]

The diverse mechanisms of action of HCTs are have not yet been fully defined.[24-32] It is known, however, that their biological activities are a result of the ability to bind transition metals. The nitrogen atom in the heterocycle along with the imine and thio atoms of the thiosemicarbazone pharmacophores form coordination complexes with the metal ions. HCTs bind particularly well

with copper, which can increase or decrease their activity. For instance, physiological concentrations of copper in human plasma (11-18 μM) strongly antagonizes 3-AP activity against RNR, while copper potentiates the cytotoxicities of Dp44mT (**2-10)** and NSC-31972632 (**2-11**) against glioblastoma and other cancers (Figure 2-2).[33-37] The ability of HCTs to bind copper increases their potential as anticancer agents, as cancers have higher intracellular levels of copper compared to healthy cells.[39,40] Using small molecules to disrupt copper homeostasis in cancers through sequestration of copper by chelation or by increasing intracellular copper concentration by acting as ionophores are established therapeutic strategies.[38,41]

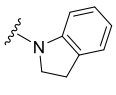
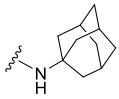
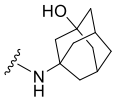
In this chapter, we report the development of pyridine- and isoquinoline-based HCTs as anticancer agents. In the latter category, we identify a lead compound which is a potent antiproliferative in the presence of physiologically relevant levels of copper, and explore the mechanism of action of this compound.

RESULTS AND DISCUSSION



Scheme 2-1. Synthesis of 3-AP (**2-5**) analogs **2-41 - 48**.

Table 2-1. 4’ amine substituents of 3-AP (**2-5**) analogs.

Compound	**2-15**	**2-16**	**2-17**	**2-18**	**2-19**	**2-20**	**2-21**	**2-22**	
	2-24	**2-25**	**2-26**	**2-27**	**2-28**	**2-29**	**2-30**	**2-31**	
	2-32	**2-33**	**2-34**	**2-35**	**2-36**	**2-37**	**2-38**	**2-39**	**2-40**
	2-41	**2-42**	**2-43**	**2-44**	**2-45**	**2-46**	**2-47**	**2-48**	3-AP **(2-5)**
									

3-AP is the most clinically successful HCT RNR inhibitor, and yet it has not progressed beyond phase II clinical trials due to toxicity and poor pharmacokinetic properties.[42,43] We set out to make analogs of 3-AP that retain potency but perform better *in vivo*. The thiosemicarbazone pharmacophore of HCTs is vital for their activity, and is not tolerant to changes, so our analogs retained that part of the structure unchanged. The 3-aminopyridine moiety is also fairly intolerant to substitution.[4] Alkylation at the 4’ nitrogen has been shown to potentiate the activity of 3-AP, so we decided to focus our efforts on substituting that position with various amines.[44] We synthesized eight analogs of 3-AP with substitution of the 4’ nitrogen. The synthesis is shown in Scheme 2-1. In line I, the amine of 3-amino-2-bromopyridine (**2-12**) was Boc-protected to form carbamate **2-13**, which was then formylated at the 2-position using *N*-formylpiperidine and *n*-butyllithium to give aldehyde **2-14**. In line II, various amines **2-15 - 22** were treated with carbon disulfide and sodium hydroxide then sodium chloroacetate **2-23** to give carbamodithioates **2-24 - 31**, which were then reacted with hydrazine hydrate to form thiosemicarbazides **2-32** – **39 (**Scheme 2-1, Table 2-1**)**. In line III, aldehyde **2-14** and the desired thiosemicarbazide **2-32 - 39** were condensed with concurrent deprotection of the amine under acidic conditions to form HCTs **2-41 - 48**.

In compounds **2-41**, **2-42**, and **2-43**, we chose cyclic amines azetidine, pyrrolidine, and piperidine respectively to increase hydrophobicity and see if there is a trend correlating ring size and activity. Morpholine as in compound **2-44** was chosen to increase the solubility, and 2-

morpholinoethan-1-amine as in compound **2-48** for the same reason but to also keep bulk farther away from the reaction center. Indoline analog **2-45** was synthesized to increase the lipophilicity and to test the effect of conjugation of the 4'-nitrogen with an aromatic ring. 3-AP (**2-5**) is planar, and cyclic amine substitutions add only a small amount of three dimensionality. We were curious as to the effect of adding a bulky adamantyl group, which would also add bulk out of the plane. Adamantyl groups are present in a number of pharmaceutical agents on the market, and they have been shown to affect the Absorption, Distribution, Metabolism, or Excretion (ADME) properties of small-molecule drugs.[45] We synthesized **2-46** to see if the ADME properties of 3-AP would be affected as well; since the adamantly group is very hydrophobic and could decrease solubility, we also synthesized adamantanol derivative **2-47**.

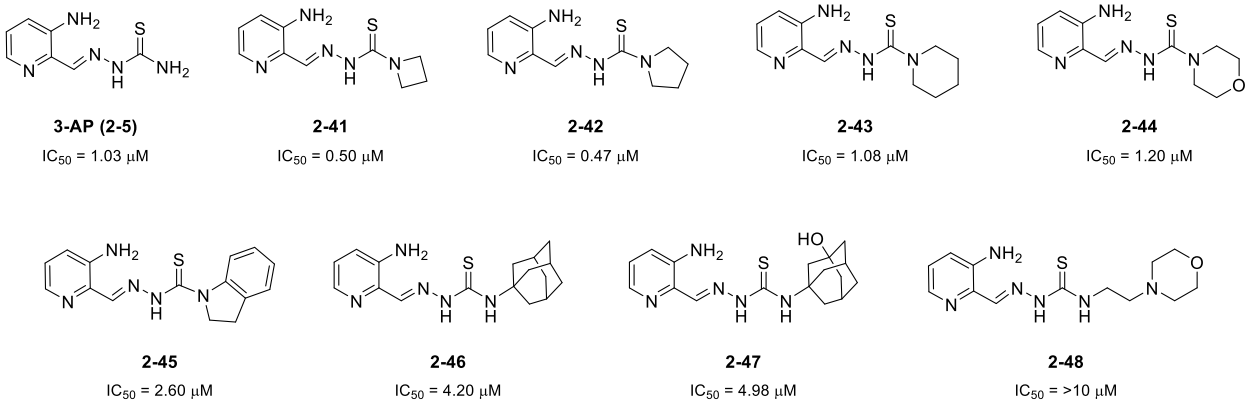


Figure 2-3. Activity of 3-AP analogs. MIAPACA2 cells were treated with the indicated HCT for 72 h, then cell viability was measured with CellTiter-Glo to determine IC_{50} values.

The compounds were tested in MIAPACA2, a pancreatic ductal adenocarcinoma (PDAC) cell line, and IC_{50} values were determined after 72 hour treatment (Figure 2-3). The series of cyclic amine analogs **2-41** through **2-43** all showed comparable or better activity than 3-AP with the 4-

and 5- membered rings being the most active, having IC_{50} values of 0.5 µM and 0.47 µM respectively. Interestingly, the analogs with primary amine substitutions were all less active than 3-AP. **2-45** was 2.5-fold less active, possibly due to decreased electron density on the thiosemicarbazone core as a result of the conjugations between the nitrogen and the aromatic ring. Adamantly analogs **2-46** and **2-47** were very insoluble and were both less active than 3-AP. It is possible that these compounds would be more active if a suitable formulation was found, but we did not explore any as they were even poorly soluble in DMSO. **2-48** was almost completely inactive. **2-42** had the lowest IC_{50} and so was chosen as the lead compound to go forward with further testing.

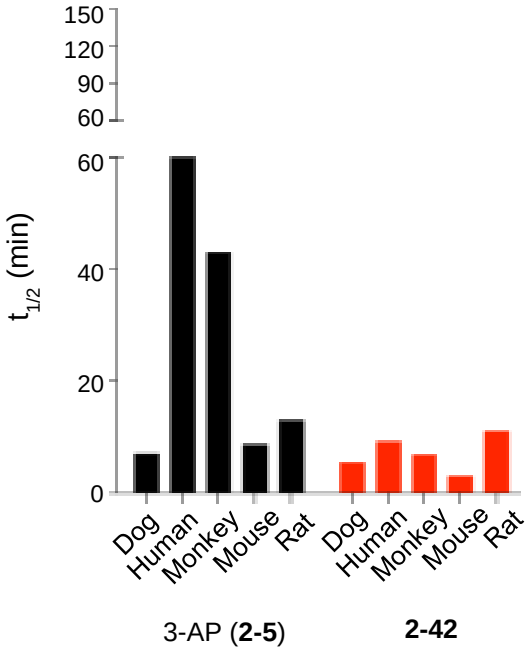
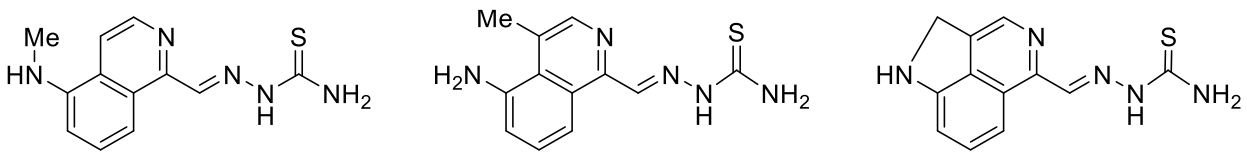


Figure 2-4. Liver microsomal stability of 3-AP (**2-5**) and **2-42**.

One of the likely reasons for the inefficacy of 3-AP against solid tumors is rapid metabolic breakdown of the drug. In clinical trials, the half life of 3-AP is typically seen to be less than 1 hour, which is consistent with our results and others.[46] The lack of drug metabolites present in

urine suggest metabolism via the liver.[47] To test the metabolic stability of our lead compound **2-42** relative to 3-AP (**2-5**), a liver microsomal assay was performed (Figure 2-4). 3 μM of drug was incubated with NADPH and 0.5 mg/mL liver microsomes from dog, human, monkey, mouse, and rat cells. Drug concentration was analyzed by LC-MS/MS. Unfortunately, **2-42** had even shorter half life in all species, under 20 minutes, meaning that its pharmacokinetic properties would be worse than 3-AP. Compounds **2-41** and **2-43** also had a similar or better activity than 3-AP but were not pursued as backup compounds as based on the similarity of the structures to **2-42**, they would likely be similarly metabolically unstable.

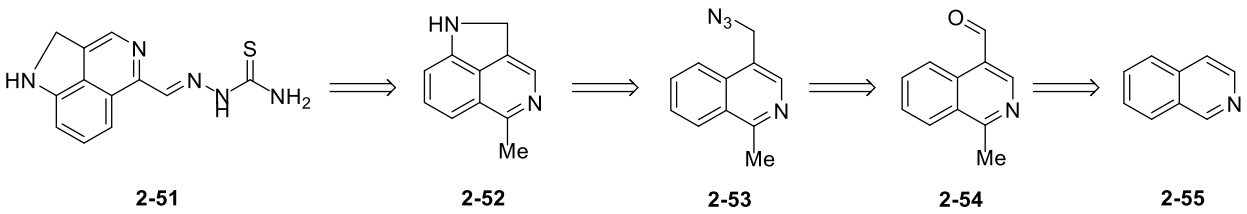


2-49 MAIQ (**2-50**) **2-51**

Figure 2-5. Compounds **2-49** and MAIQ (**2-50**), and proposed derivative **2-51**.

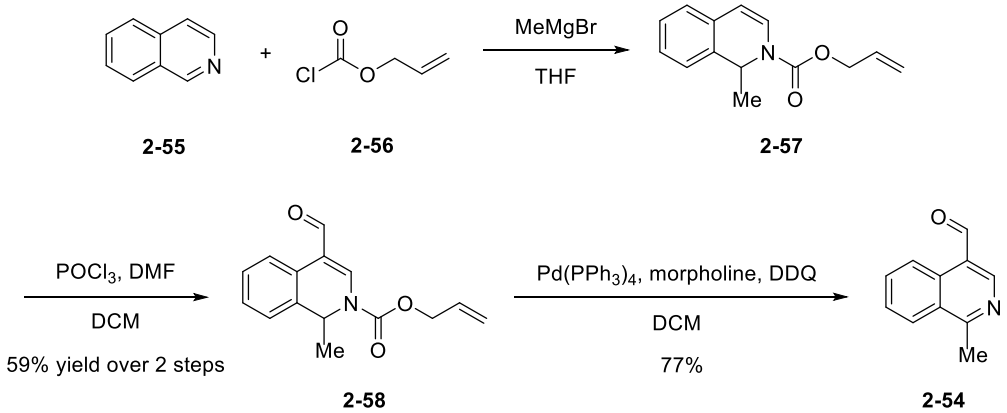
Because of the lack of success of our pyridine HCTs, we revisited the isoquinoline class of HCTs which have shown very promising activity.[3] Isoquinoline HCTs have been extensively studied but new research on them has slowed down considerably since the development of 3-AP in 1992.[4] One promising isoquinoline HCT with a 5-methylamine substitution, **2-49** in Figure 2-5**,** was shown to increase the life span of tumor-bearing mice 2.5-fold at a dose of 40 mg/kg/day, but was not pursued clinically.[48] Its isomer, 5-amino-4-methyl-1-formylisoquinoline thiosemicarbazone, or MAIQ (**2-50**) is also active but was similarly abandoned.[5] We proposed a tricyclic analog of these compounds, with the structure created by forming a 5-membered ring using the nitrogen from the amine at the 5-position and the methyl group from either structure

becoming a methylene bonded to the nitrogen and the 4-postion (**2-51**). We hypothesized that closing the ring would make the structure more rigid and thus more stable. The compound would also be a completely novel structure in the well-explored patent space of HCTs.



Scheme 2-2. Retrosynthesis of tricyclic HCT **2-51**.

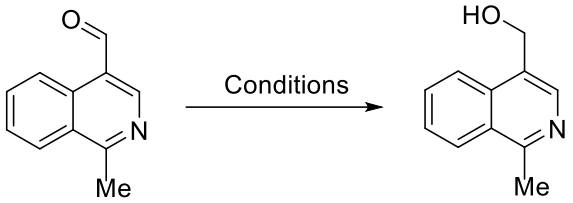
The retrosynthesis of the tricyclic drug is shown in Scheme 2-2. The final compound **2-51** could be synthesized from 1-methylisoquinoline derivative **2-52** through oxidation of the methyl group to the aldehyde then condensation with thiosemicarbazide (**2-40**). The tricyclic core **2-52** was envisioned to be formed through C-H insertion at the 5-position by the nitrene species arising from azide **2-53.** The azide could be formed from aldehyde **2-54** through its reduction product, the corresponding primary alcohol. Isoquinoline **(2-55)** could be transformed into aldehyde **2-54** through methylation at the 1-position followed by Vilsmeier-Haack formylation.



Scheme 2-3. Synthesis of aldehyde **2-54** from isoquinoline (**2-55**).

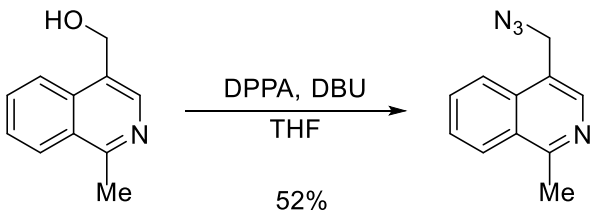
To begin the synthesis of the tricyclic core, isoquinoline (**2-55**) and allyl chloroformate (**2-56**) were treated with methylmagnesium bromide as shown in Scheme 2-3 to affect the addition of the methyl group to the 1-position, giving **2-57.** This was followed by a Vilsmeier-Haack reaction which gave the aldehyde **2-58**. Treatment with tetrakis(triphenylphosphine)palladium(0), morpholine, and DDQ resulted in the reoxidized isoquinoline **2-54**.

Table 2-2. Reduction of aldehyde **2-54** to alcohol **2-59**.



2-54 **2-59**

Entry	Conditions	Result
1	$NaBH_4$, 1 eq	N.R.
2	$NaBH_4$, excess	N.R.
3	$NaBH_4$, excess, 40 ˚C	N.R.
4	H_2, Pd/C, 3 atm	Complex mixture/ N.R.
5	DIBAL-H	N.R.
6	$NaBH_4$, $CeCl_3$•$7H_2O$	89% yield

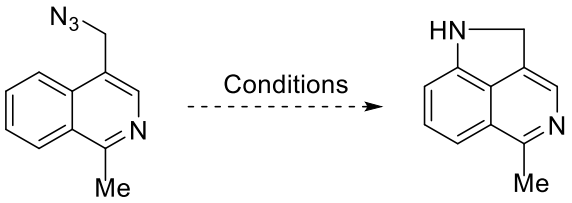


2-59 **2-53**

Scheme 2-4. Conversion of alcohol **2-59** to azide **2-53**.

The reduction of the aldehyde **2-54** to alcohol **2-59** was expected to proceed easily, however many standard reduction conditions did not result in the desired product as shown in Table 2-2. In entries 1-3, treating **2-59** with sodium borohydride in either 1 equivalent, excess at room temperature, or excess at 40 ˚C resulted in no reaction. Treatment with hydrogen and palladium on carbon at 3 atmospheres gave a mixture of starting material and many other products. Treatment with diisobutylaluminum hydride also gave no reaction. We then found that Luche reduction conditions furnished the alcohol in 89% yield. In Scheme 2-4, the alcohol **2-59** was converted to the azide **2-53** with diphenylphosphoryl azide and DBU in 52% yield.

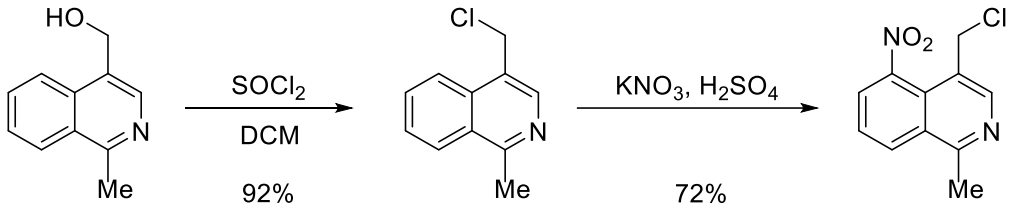
Table 2-3. Attempted intramolecular cyclization of **2-53** to **2-52**.



2-53 **2-52**

Entry	Conditions	Result
1	$AlCl_3$, DCM, RT	N.R.
2	$AlCl_3$, DCM, reflux	Side product
3	$Rh_2(OAc)_4$, toluene	N.R.
4	1,2-dichlorobenzene, 160 ˚C	N.R.
5	H_2SO_4, 140 ˚C	Side product
6	Tetralin, MW, 300W, 200 ˚C	Side product
7	AcOH, toluene, MW, 300W, 120 ˚C	N.R.

We then attempted cyclization of the azide **2-53** to the tricycle **2-52** via intramolecular C-H insertion. Treatment with aluminum trichloride at room temperature gave no reaction, while the same conditions under reflux resulted in an unidentified side product with a mass of 159.0912 [M^+ + H].[49] No reaction was observed when **2-53** was treated with rhodium diacetate. Heating **2-53** in 1,2-dichlorobenzene at 160 ˚C also gave no reaction.[50] Heating **2-53** in sulfuric acid at 140 ˚C gave the unidentified side product, as did microwaving at 300 W at 200 ˚C in tetralin.[51] Treatment with acetic acid in toluene under microwave conditions gave no reaction.

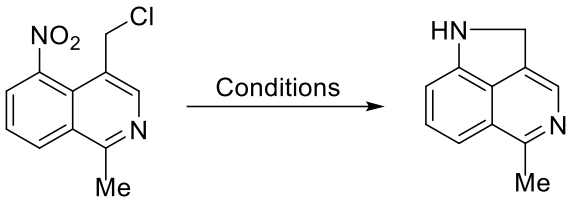


2-59 **2-60** **2-61**

Scheme 2-5. Synthesis of isoquinoline **2-61**.

Because of the negative results of the C-H insertion reaction screen, we revised our forward synthesis starting with alcohol **2-59.** Chlorination to **2-60** then nitration would give **2-61** as in Scheme 2-5, which could then be reduced in order to undergo an internal substitution to form the 5-membered ring of **2-52** as shown in Table 2-4. Alcohol **2-59** was treated with thionyl chloride to give **2-60** in excellent yield. Potassium nitrate and sulfuric acid gave **2-61**, with nitration at the 5-position.

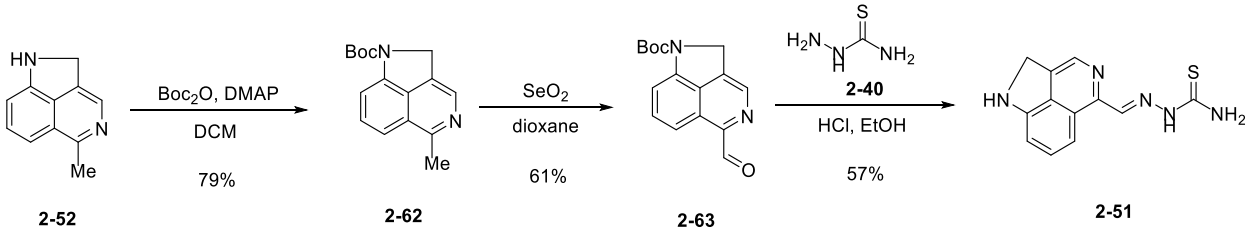
Table 2-4. Reduction-cyclization to **2-52.**



2-61 **2-52**

Entry	Conditions	Result
1	$NiCl_2•H_2O$, $NaBH_4$	Reduction, loss of Cl
2	H_2, Pd/C	Reduction, loss of Cl
3	$SnCl_2•2H_2O$, EtOH	N.R.
4	AcOH, NH_4CHO_2, Pd/C, MeOH	N.R.
5	Fe, AcOH, H_2O, xylenes	80% yield

We then proceeded to form the 5-membered ring under reducing condition as shown in Table 2-4. Many sets of conditions resulted in either under- or overreduction. Nickel chloride hydrate and sodium borohydride gave reduction of the nitro group to the amine as well as loss of the chlorine atom, as did hydrogen and palladium on carbon. Stannous chloride hydrate in ethanol resulted in no reaction. Treatment with acetic acid, ammonium formate, and palladium on carbon gave no reaction as well. It was then found that **2-61** in xylenes carefully added to iron and acetic acid in water gave the cyclized product **2-52** in 80% yield.

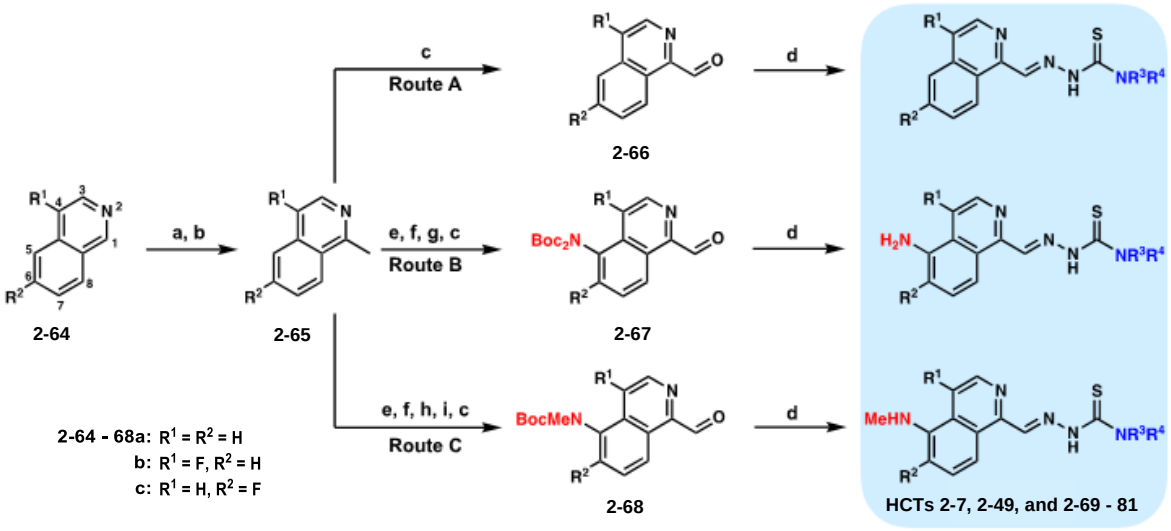


2-52 **2-62** **2-63** **2-51**

Scheme 2-6. Synthesis of HCT **2-51** from tricyclic core **2-52**.

With the tricyclic core in hand, the thiosemicarbazone was synthesized, beginning with Boc-protecting the amine of **2-52** to **2-62.** The methyl group was then oxidized to the aldehyde **2-63** with selenium dioxide. Condensation with thiosemicarbazide (**2-40**) as well as the deprotection of the amine proceeded via treatment with aqueous HCl in ethanol to give **2-51**.

2-51 was tested in MIAPACA2, and unfortunately its IC_{50} was 2.5 µM, making it less than half as active as MAIQ in our assay. Given the lack of improvement in activity and complexity of the synthesis, we decided not to pursue this tricyclic analog.



Scheme 2-7. Synthesis HCT compounds **2-7**, **2-49**, and **2-69 - 81** from simple isoquinolines[a,b,c]

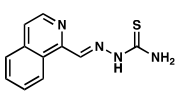
[a](a) allyl chloroformate, MeMgBr, THF; (b) $Pd(PPh_3)_4$, morpholine; DDQ, CH_2Cl_2; (c) SeO_2, 1,4-dioxane, 60 °C; (d) appropriate thiosemicarbazide, HCl, EtOH, reflux or microwave 50 °C; (e) KNO_3, H_2SO_4; (f) Fe, HCl, MeOH, reflux; (g) Boc_2O, DMAP, TEA, THF; (h) Boc_2O, DMAP, TEA, THF; $NaHCO_3$, MeOH, reflux or K_2CO_3, MeOH, reflux; (i) NaH, THF; MeI. [b]2-7, 2-69, 2-70, 2-72 - 74, and 2-77 - 79 synthesized through Route A; 2-71 and 2-76 synthesized through Route B; 2-49, 2-75, 2-80, and 2-81 synthesized through Route C. [c]Intermediates not shown from Routes B and C are described in the Experimental section.

Despite these discouraging results, we were not deterred from developing other isoquinoline-based HCTs. Because of the well-established beneficial effects of fluorine on small-molecule drugs, we decided to synthesize fluorine-substituted isoquinoline HCTs.[52,53] The 5-, 7-, and 8-fluoro analogs of IQ-1 (**2-7**) are known, and variance in their potency and toxicity demonstrate that the position of the fluorine determines its effect on activity, although no trends can be observed.[5] The cytotoxicity of 3-AP analogs is potentiated by 4' amine alkylation, so we decided to methylate and dimethylate the 4' amine to see if that could possibly synergize with the ring substitutions.[31,44,54]

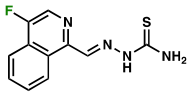
We synthesized 15 isoquinoline-based HCTs with substitutions to the heterocycle and the 4' amine (Scheme 2-7, Table 2-5). The synthesis for all compounds began with methylation of the appropriate isoquinoline **2-64** to generate **2-65** (Scheme 2-7). Depending on whether the desired 5-position substituent was hydrogen, amine, or methylamine, **2-65** was then subjected to either Route A, B or C, respectively. Compounds **2-7**, **2-69**, **2-70**, **2-72 - 74**, and **2-77 - 79** were synthesized by Route A, in which the 1-methyl substituent of **2-65** was oxidized using selenium dioxide to give the aldehyde **2-66**. Condensation with the appropriate thiosemicarbazide under acidic conditions afforded the desired HCT compound. **2-71** and **2-76** were synthesized via Route B, which began with nitration of the 5-position of **2-65** followed by an iron-mediated reduction to the amine. Subsequent Boc-protection and oxidation produced aldehyde **2-67**. This was followed by simultaneous condensation with the appropriate thiosemicarbazide and Boc-deprotection under acidic conditions, which gave the desired HCT. Syntheses of **2-49**, **2-75**, **2-80**, and **2-81** via Route C proceeded similarly as Route B from **2-65** with installation of a nitro group, reduction to the amine, and Boc-protection followed by treatment with either potassium or sodium bicarbonate to furnish the monocarbamide. The protected amine was then methylated with methyl iodide and

sodium hydride, and oxidation with selenium dioxide gave **2-68**. Concurrent condensation and Boc-deprotection were achieved under acidic conditions to provide the target HCT. With some HCTs, particularly for **2-77 – 81,** we observed the presence of a minor *Z*-isomer of the product, typically in less than 10%. This presence of this isomer is due to an intramolecular hydrogen bond between the 2' amine of the thiosemicarbazone and the isoquinoline nitrogen, forming a stable 6-membered species. The *E*- and *Z*-isomers were inseparable by HPLC purification and were used as a mixture in all assays. We did not expect a reduction in activity due to the isomer, as previous studies reported no significant difference in potency.[54]

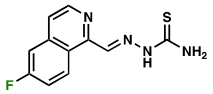
4' primary amines



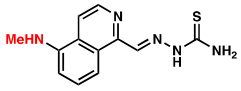
2-7
IC_{90}: 18100 nM
+Cu(II) IC_{90}: 2210 nM



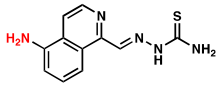
2-69
IC_{90}: 24600 nM
+Cu(II) IC_{90}: 2060 nM



2-70
IC_{90}: 5440 nM
+Cu(II) IC_{90}: 2040 nM

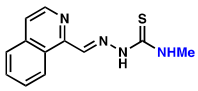


2-49
IC_{90}: 11500 nM
+Cu(II) IC_{90}: 5330 nM

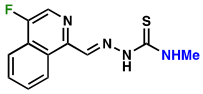


2-71
IC_{90}: 40500 nM
+Cu(II) IC_{90}:71100 nM

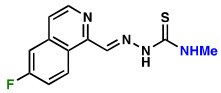
4' secondary amines



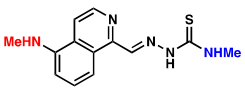
2-72
IC_{90}: 18700 nM
+Cu(II) IC_{90}: 331 nM



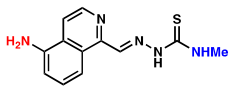
2-73
IC_{90}: 2080 nM
+Cu(II) IC_{90}: 114 nM



2-74
IC_{90}: 4080 nM
+Cu(II) IC_{90}: 233 nM

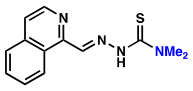


2-75
IC_{90}: 4240 nM
+Cu(II) IC_{90}: 607 nM

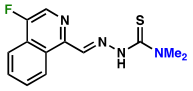


2-76
IC_{90}: 11200 nM
+Cu(II) IC_{90}:7870 nM

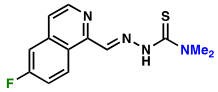
4' tertiary amines



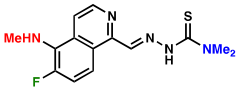
2-77
IC_{90}: 29600 nM
+Cu(II) IC_{90}: 73.7 nM



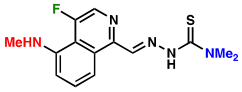
2-78
IC_{90}: 274 nM
+Cu(II) IC_{90}: 26.6 nM



2-79
IC_{90}: 111 nM
+Cu(II) IC_{90}: 21.6 nM



2-80
IC_{90}: 272 nM
+Cu(II) IC_{90}: 38.4 nM



2-81
IC_{90}: 327 nM
+Cu(II) IC_{90}: 42.3 nM

Figure 2-6. Cu(II) supplementation potentiates the activity of isoquinoline HCTs. MIAPACA2 cells were treated with the indicated HCT ± 20 µM Cu(II) for 72 h, then cell viability was measured with CellTiter-Glo to determine IC_{90} values.

Table 2-5. Substitution patterns of compounds **2-7**, **2-49**, and **2-69 - 81**.

	2-7	**2-69**	**2-70**	**2-49**	**2-71**	**2-72**	**2-73**	**2-74**	**2-75**	**2-76**	**2-77**	**2-78**	**2-79**	**2-80**	**2-81**
R^1	H	F	H	H	H	H	F	H	H	H	H	F	H	H	F
R^2	H	H	F	H	H	H	H	F	H	H	H	H	F	F	H
R^3	H	H	H	H	H	Me	Me	Me	Me	Me	Me	Me	Me	Me	Me
R^4	H	H	H	H	H	H	H	H	H	H	Me	Me	Me	Me	Me
5-pos	H	H	H	NHMe	NH_2	H	H	H	NH_2	NH_2	H	H	H	NHMe	NHMe

We first tested our compounds in MIAPACA2 cells using conventional cell culture conditions (DMEM media and 10% FBS) and determined IC_{90} values (Figure 2-6). Compounds were divided into three categories based on sequential methylation– non-methylated 4' primary amines, monomethylated 4' secondary amines, and dimethylated 4' tertiary amines. Among the first series, **2-7**, **2-49**, and **2-71** are known compounds and were included as controls against which to measure the effect of fluorine substitution.[55,56] Compared to the unsubstituted analog, the 4-fluoro derivative **2-69** did not show increased potency, while the 6-fluoro analog **2-70** was 3-fold more potent. This demonstrates that fluorination on the isoquinoline does alter the potency of HCTs, and the effect is position-dependent.

In the 4' secondary amine series, a synergistic effect was observed when combining substitution on the isoquinoline with 4' amine methylation. HCTs **2-73 - 76** were all more potent than the unsubstituted analog **2-72**, and were also all more potent than the corresponding compounds in the primary 4' amine series. This effect was especially pronounced in **2-73.**

The effect of fluorine substitution amplified among the dimethylated 4'-tertiary amine compounds **2-78** and **2-79**, which had nanomolar IC_{90} values. Fluorination at the 4-position resulted in roughly 110-fold increased potency relative to the non-fluorinated analog **2-77**, while the analog fluorinated at the 6-position, **2-79**, was 270-fold more potent. Similarly, the fluorinated

5-methylamino compounds **2-80** and **2-81** also showed synergy between 4' methylation and fluorination, with the 6-position fluorination leading to the more active compound.

HCTs are known to chelate copper strongly, and it was recently shown that activity of NSC319726 (**2-11**), a 4' tertiary amine HCT, against glioblastoma increased dramatically with copper supplementation.[38] To see if our compounds would also be potentiated by copper, we determined IC_{90} (+Cu(II) IC_{90} in Figure 2-6) values against MIAPACA2 cells in media supplemented with physiologically relevant concentrations of copper (DMEM media + 10% FBS + 20 μM $CuCl_2$) (Fig. 2-6). All compounds with the exception of **2-71** showed significant potentiation of activity with copper supplementation. For unsubstituted isoquinoline compounds **2-7**, **2-72**, and **2-77**, a trend emerged where the fold change in potency upon addition of copper increased with sequential methylation, i.e. **2-7** was 8 times more potent with copper than without, **2-72** was 56-fold more potent, and **2-77** was 402-fold more potent. The trends of increasing activity with addition of copper and with each sequential methylation of the 4' amine held for the fluorinated analogs as well. Compounds **2-78 - 81** are all 5- to 10-fold more active with copper supplementation than without. **2-78** and **2-79** are both more potent than the corresponding 4' secondary amine compounds, which are in turn more active than the 4' primary amine compounds. The 4' dimethylamine compounds are all very active, having IC_{90} values in the mid nanomolar range, with **2-79** being the most active at 21.6 nM. Additionally, fluorine substitution at both the 4- and 6-positions led to greater potency when compared with corresponding non-fluorinated analogs. These results indicate that potency increases synergistically with both fluorination of the isoquinoline and methylation of the 4' amine, and that the activity of isoquinoline HCTs was potentiated by supplementation with physiologically relevant levels of copper. Because of its

potency and relatively straightforward synthesis, **2-79** was chosen as the lead compound for studies into the mechanism of action.

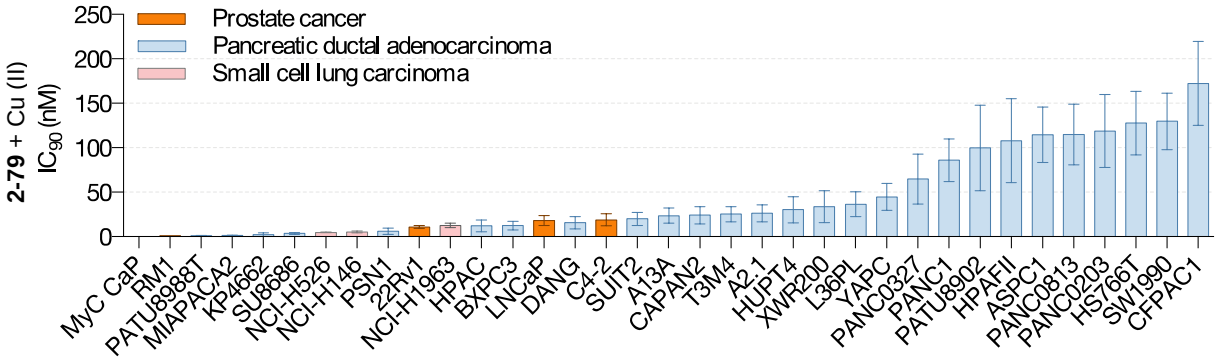


Figure 2-7. IC_{90} of **2-79** in a panel of human and mouse prostate cancer (PC), small cell lung carcinoma (SCLC) and pancreatic ductal adenocarcinoma (PDAC) models treated with **2-79** + Cu(II) (20 µM) for 72 h measured by CellTiterGlo.

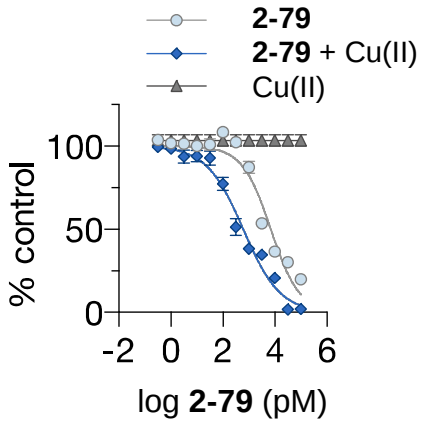


Figure 2-8. Proliferation of MIAPACA2 PDAC cells measured by CellTiterGlo following **2-79** treatment for 72 h ± Cu(II) (20 µM), and with Cu(II) alone.

Elevated serum copper levels (>20 µM) are observed in patients with solid tumor types such as pancreatic ductal adenocarcinoma (PDAC), small cell lung carcinoma (SCLC), and prostate cancer (PC).[56-61] These cancers rely upon elevated levels of copper for growth and metastasis, making it a viable therapeutic target.[40] In a panel of PDAC, SCLC, and PC cancer models cultured in media supplemented with physiologically relevant concentrations of copper (20 µM $CuCl_2$), **2-79** was very potent, with +Cu IC_{90} values ranging from 1 nM to 200 nM (Figure 2-7).

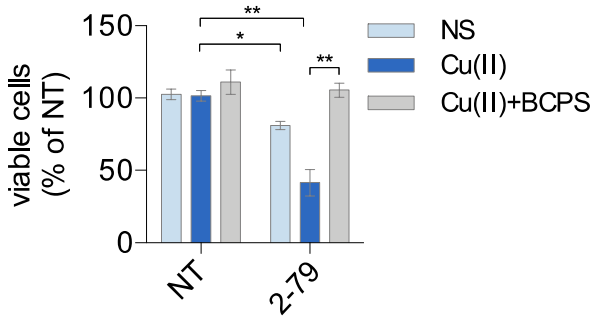


Figure 2-9. Inhibition of proliferation of MIAPACA2 cells treated with **2-79** (25 nM) + Cu(II) (20 µM) for 24 h ± bathocuproine disulfonate (BCPS, 300 µM) measured by trypan blue exclusion.

(mean ± SD, n = 2, one-way ANOVA corrected for multiple comparisons by Bonferroni adjustment, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

We used the MIAPACA2 cell line, a PDAC model that was highly sensitive to **2-79**, to probe the mechanism of action of the drug. In Figure 2-8, proliferation of MIAPACA2 cells was measured in response to **2-79** with and without supplementation with 20 µM Cu(II) as well as in response to 20 µM Cu(II) alone, using $CuCl_2$ as a source of Cu(II). Supplementation with Cu(II) led to the IC_{90} of **2-79** decreasing from 110 nM to 21 nM. Without **2-79** treatment, Cu(II)

supplementation had no effect on proliferation. To confirm that copper is indeed responsible for the cytotoxicity of **2-79**, we combined treatment with bathocuproine disulfonate (BCPS), a membrane impermeable Cu(II) chelator, with **2-79** in the presence of Cu(II), and growth inhibition was completely negated (Figure 2-9). This suggests that cytotoxicity of **2-79** is dependent on available copper.

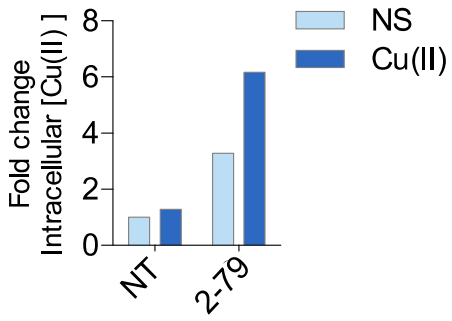


Figure 2-10. Intracellular concentrations of copper measured by inductively coupled plasma mass spectrometry (ICP-MS) in MIAPACA2 cells treated with **2-79** (25 nM) for 24 h ± Cu(II) (20 µM).

Since the effect of **2-79** seemed to be copper-dependent, we hypothesized that the activity would benefit from increased copper concentration, which could occur if **2-79** acted as an ionophore and transported copper into cells. To test whether this was the case for **2-79,** we measured intracellular copper levels using inductively coupled plasma mass spectrometry (ICP-MS). As shown in Figure 2-10, intracellular copper levels increased in the presence of **2-79** alone, and to a greater extent with **2-79** and Cu(II) supplementation, suggesting that it is an ionophore for copper.

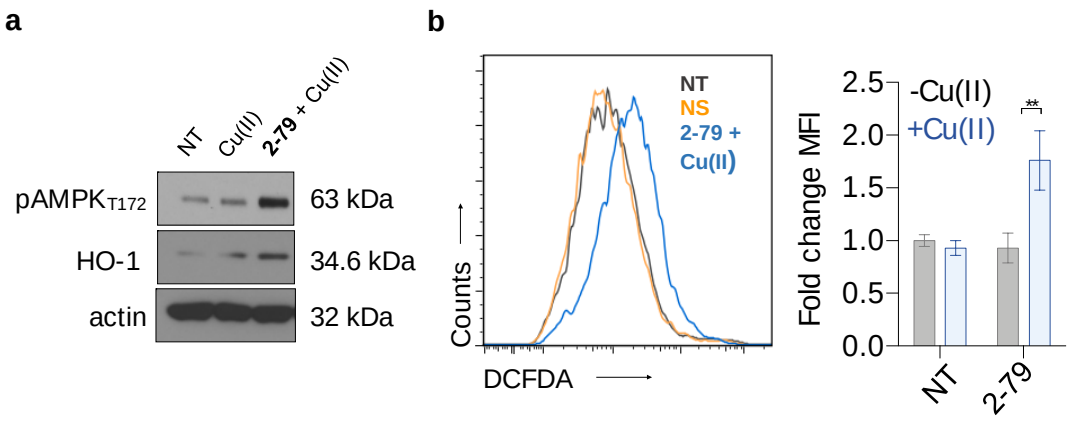


Figure 2-11. Induction of ROS by **2-79**. (**a**) Representative immunoblots of MIAPACA2 cells treated as indicated for 24 h. (**b**) Reactive oxygen species (ROS) measurement using CM-H2DCFDA staining after **2-79** (25 nM) + Cu(II) (20 µM) treatment for 24 h. (mean ± SD, n = 2, Student t-test, ***P < 0.001).

We consistently observed induction of AMPK phosphorylation (T172) at 24 h in cells treated with **2-79** with Cu(II)-supplementation, demonstrating suppression of mitochondrial oxidative phosphorylation (Figure 2-11a). Additionally, heme oxygenase-1 (HO-1) levels increased with **2-79** treatment, indicating ROS induction (Figure 2-11a). To confirm whether **2-79** treatment was leading to ROS generation, we used CM-H2DCFDA staining, and found that there was an almost two-fold increase in the amount of detectable ROS generated with copper supplementation than without (Figure 2-11b).

CONCLUSION

Pyridine- and isoquinoline-based HCTs have been synthesized and evaluated for antiproliferative activity. A series of 3-AP analogs was synthesized, and while some retained 3-AP's potency, none improved on its drug-like properties enough to be a promising drug candidate. A series of isoquinoline-based HCTs was also synthesized, several of which synergize strongly with physiologically relevant levels of Cu(II) supplementation. The lead compound 6-fluoroisoquinoline-1-carboxaldehyde *N*,*N*-dimethylthiosemicarbazone (**2-79**) was potent against several solid tumor cell lines, and we showed that the cytotoxicity is copper dependent. This compound is a promising antiproliferative in cancer and warrants further investigation.

EXPERIMENTAL

General

All chemicals, reagents and solvents were obtained from commercial sources and were used without further purification. Unless otherwise noted, reactions were carried out in oven-dried glassware under an atmosphere of argon using commercially available anhydrous solvents. Tetrahydrofuran (THF) was distilled from sodium under an argon atmosphere. Dichloromethane was distilled from calcium hydride. Solvents used for extractions and chromatography were not anhydrous. Analytical TLC was carried out on precoated silica gel (Merck silica gel 60, F254) and visualized with UV light. Column chromatography was performed with silica (Fisher, 230-400 mesh). ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra were measured in $CDCl_3$ or DMSO-d_6 on Bruker AV spectrometers at 300, 400 or 500 MHz. Chemical shifts were reported in parts per million (δ) relative to residual solvent signals. The signals observed were described as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), qd (quartet of doublets), ddd (doublet of doublet of doublets), tdd (triplet of doublet of doublets), ddt (doublet of doublet of triplets), m (multiplet), br s (broad singlet), appbr s (apparent broad singlet). Mass spectra were obtained on a Waters LCT Premier with ACQUITY UPLC mass spectrometer under electrospray ionization (ESI) or Thermo Fisher Scientific Exactive Plus with direct analysis in real time (DART) ionization. Purity of all compounds used in biological assays was determined on a Hewlett Packard 1090 HPLC system using an Aquasil C18 column (250 mm × 2 mm, 5 µm, Keystone Scientific) with an acetonitrile/water solvent system containing 0.1% TFA with detection performed at 254 nm. HPLC purification was performed on a Hewlett Packard 1090 HPLC system with Hypersil Gold column (250 mm × 10 mm, 5 µm, Thermo Scientific) with and acetonitrile/water solvent system containing 0.05% formic acid and 10 mM ammonium formate.

All microwave-assisted reactions were carried out in a CEM Discover 908005 Microwave synthesizer system.

***tert*-Butyl-(2-bromopyridin-3-yl)carbamate (2-13).** To a solution of Boc_2O (30.26 g, 138.72 mmol) in THF (50 mL) was added DMAP (0.565g, 4.62 mmol). The mixture was stirred at 0 ˚C for 10 minutes, and 3-amino-2-bromopyridine (8.00 g, 46.2 mmol) was added. The mixture was refluxed for 6 h and cooled to room temperature. A solution of K_2CO_3 (19.17g, 138.72 mmol) in methanol (50 mL) was added and the mixture was refluxed for 2 h, then cooled, filtered, and concentrated. The crude residue was purified by silica gel column (15–25% ethyl acetate/hexanes) to afford the carbamate **2-13** as a beige solid (10.86 g, 86% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ

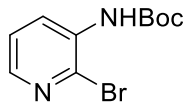
8.74 (s, 1H)

8.16 (dd, J = 4.6, 1.8 Hz, 1H)

7.92 (dd, J = 7.9, 1.8 Hz, 1H)

7.42 (dd, J = 8.0, 4.6 Hz, 1H)

1.46 (s, 9H).



2-13

^{13}C NMR (100 MHz, DMSO-d_6) δ 152.92, 145.82, 137.83, 134.30, 134.11, 123.56, 79.97, 27.97.

DART-MS: *m/z* calcd for $C_{10}H_{14}BrN_2O_2$ $(M+H)^+$ 273.02332, found 273.02267.

***tert*-Butyl-(2-formylpyridin-3-yl)carbamate (2-14).** To a solution of the bromide **2-13** (2.00 g, 7.32 mmol) in THF (20 mL) was added *n*-butyllithium (1.6M, 9.15 mL, 14.65 mmol) and the mixture was stirred for 1 h at -78 ˚C. The mixture was warmed to 0 ˚C and *N*-formylpiperidine (0.89 mL, 8.05 mmol) was added and stirring commenced for 1 h. The solution was quenched with 1.0 M HCl (15 mL) then neutralized with solid K_2CO_3. The aqueous layer was extracted with ethyl acetate (3 x 10 mL) and the combined organic layers were washed with brine then dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (0–15% ethyl acetate/hexanes) to yield the aldehyde **2-14** as an off-white solid (1.18 g, 72% yield).

^{1}H NMR (400 MHz, Chloroform-*d*) δ

10.21 (s, 1H)

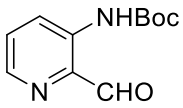
10.07 (d, *J* = 0.8 Hz, 1H)

8.83 (dt, *J* = 8.7, 1.1 Hz, 1H)

8.40 (dd, *J* = 4.4, 1.4 Hz, 1H)

7.44 (ddd, *J* = 8.7, 4.4, 0.6 Hz, 1H)

1.53 (s, 9H).



2-14

^{13}C NMR (100 MHz, Chloroform-*d*) δ 197.20, 152.76, 143.31, 139.28, 136.72, 128.67, 126.28, 81.69, 28.25.

DART-MS: *m/z* calcd for $C_{11}H_{15}N_2O_3$ $(M+H)^+$ 223.10772, found 223.10701.

2-((3-Aminopyridin-2-yl)methylene)hydrazine-1-carbothioamide (2-5). To a solution of the aldehyde **2-14** (0.90 g, 4.05 mmol) and thiosemicarbazide (0.406 g, 4.45 mmol) in ethanol (10 mL) was added 6 M HCl (3.67 mL). The mixture was refluxed for 2 h then cooled to room temperature and the resulting precipitate was filtered off and suspended in water (10 mL). A 10% $NaHCO_3$ solution (10 mL) was added and the mixture was stirred for 1 hour then filtered, and the solid was washed successively with water, ethanol, and diethyl ether. The thiosemicarbazone **2-5** was obtained as a yellow solid (0.704 g, 89% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

11.29 (s, 1H)

8.32 (s, 1H)

8.16 (s, 1H)

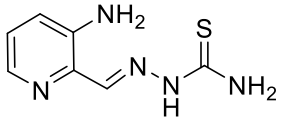
7.96 (s, 1H)

7.82 (dd, J = 4.3, 1.4 Hz, 1H)

7.14 (dd, J = 8.3, 1.5 Hz, 1H)

7.06 (dd, J = 8.3, 4.3 Hz, 1H)

6.44 (s, 2H).



2-5

^{13}C NMR (125 MHz, DMSO-d_6) δ 177.07, 149.20, 143.97, 137.11, 132.79, 124.43, 122.21.

DART-MS: *m/z* calcd for $C_7H_{10}N_5S$ $(M+H)^+$ 196.06514, found 196.06479.

2-((Pyrrolidine-1-carbonothioyl)thio)acetic acid (2-25). To a solution of pyrrolidine (200 mg, 2.81 mmol) and sodium hydroxide (118 mg, 2.95 mmol) in ethanol (2 mL) was added dropwise carbon disufide (0.169 mL, 2.81 mmol). The mixture was stirred for 4 h then cooled to 0º C. Sodium chloroacetate (0.327 g, 2.81 mmol) in water (1 mL) was added. The mixture was allowed to warm to room temperature and stirred for 12 h. The solution was acidified to pH 2 with HCl (12 M in H_2O), and a white precipitate formed which was filtered and washed with 1.2 M HCl and water. The product was recrystallized from ethanol to yield the carbamodithioate **2-25** as a white crystalline solid (0.456 g, 79% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

12.72 (s, 1H)

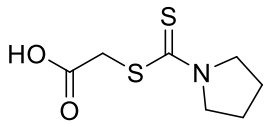
4.11 (s, 2H)

3.75 (t, J = 7.0 Hz, 2H)

3.64 (t, J = 6.9 Hz, 2H)

2.04 (p, J = 6.8 Hz, 2H)

1.92 (p, J = 6.6 Hz, 2H).



2-25

^{13}C NMR (125 MHz, DMSO-d_6) δ 190.06, 169.38, 55.22, 50.54, 38.12, 25.66, 23.80.

DART-MS: *m/z* calcd for $C_7H_{12}NO_2S_2$ $(M+H)^+$ 206.03040, found 206.02979.

Pyrrolidine-1-carbothiohydrazide (2-33). The carbamodithioate **2-25** (0.229 g, 1.12 mmol) was suspended in H_2O (2 mL), and hydrazine hydrate (0.272 mL, 5.60 mmol, 80% in H_2O) was added. The solution was heated at 50 ºC for 1 h, at which point a white precipitate formed and the reaction

was cooled to room temperature. The precipitate was filtered, washed with water, and recrystallized from ethanol to yield the thiosemicarbazide **2-33** as a white crystalline solid (0.112 g, 69% yield).

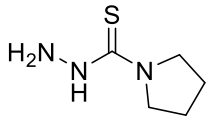
^{1}H NMR (500 MHz, DMSO-d_6) δ

8.57 (s, 1H)

4.59 (s, 2H)

3.45 – 3.40 (m, 4H)

1.88 – 1.80 (m, 4H).



2-33

^{13}C NMR (125 MHz, DMSO-d_6) δ 179.68, 49.28, 24.69.

DART-MS: *m/z* calcd for $C_5H_{12}N_3S$ $(M+H)^+$ 146.07464, found 146.07411.

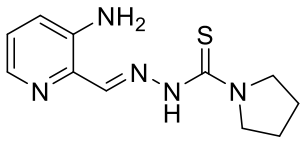
***N*'-((3-Aminopyridin-2-yl)methylene)pyrrolidine-1-carbothiohydrazide (2-42).** The aldehyde **2-14** (88.9 mg, 0.40 mmol) and the thiosemicarbazide **2-33** (58.0 mg, 0.40 mmol) were dissolved in ethanol (1 mL) and the mixture was refluxed for 4 h. The precipitate was collected by filtration and washed with cold ethanol. The crude solid was dissolved in chloroform (2 mL) and trifluoroacetic acid (0.15 mL, 2.0 mmol) was added dropwise. The mixture was stirred for 1 h and sat. $NaHCO_3$ was added to reach pH 9. A yellow precipitate formed immediately and the mixture was stirred for 1 h. The solid was collected by filtration, washed with water, and recrystallized from ethanol to yield the thiosemicarbazide **2-42** as a yellow solid (0.063 g, 63% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

11.00 (s, 1H)

8.53 (s, 1H)

7.82 (dd, *J* = 4.2, 1.5 Hz, 1H)



7.18 (s, 2H)

7.06 (qd, *J* = 8.4, 2.9 Hz, 2H) 2-42

3.64 (s, 4H)

1.93 (s, 4H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 176.04, 148.21, 143.63, 136.52, 134.01, 123.79, 121.85, 32.22.

DART-MS: *m/z* calcd for $C_{11}H_{16}N_5S$ $(M+H)^+$ 250.11209, found 250.11212.

2-((Piperidine-1-carbonothioyl)thio)acetic acid (2-26). To a solution of piperidine hydrochloride (200 mg, 1.65 mmol) and sodium hydroxide (138 mg, 3.45 mmol) in ethanol (2 mL) was added dropwise carbon disufide (0.099 mL, 1.65 mmol). The mixture was stirred for 4 h then cooled to 0º C. Sodium chloroacetate (0.192 g, 1.65 mmol) in water (1 mL) was added. The mixture was allowed to warm to room temperature and stirred for 12 h. The solution was acidified to pH 2 with HCl (12 M in H_2O), and a white precipitate formed which was filtered and washed with 1.2 M HCl and water. The product was recrystallized from ethanol to yield the carbamodithioate **2-26** as a white crystalline solid (0.277 g, 77% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ

12.70 (s, 1H)

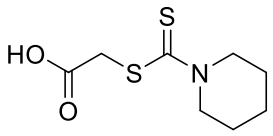
4.20 (s, 2H)

4.11 (s, 2H)

3.91 (s, 2H)

1.72 – 1.62 (m, 2H)

1.59 (s, 4H).



2-26

^{13}C NMR (125 MHz, DMSO-d_6) δ 193.01, 169.42, 52.70, 51.07, 38.69, 25.87, 25.22, 23.53.

DART-MS: *m/z* calcd for $C_8H_{14}NO_2S_2$ $(M+H)^+$ 220.04605, found 220.04581.

Piperidine-1-carbothiohydrazide (2-34). The carbamodithioate **2-26** (0.269 g, 1.23 mmol) was suspended in H_2O (2 mL), and hydrazine hydrate (0.30 mL, 6.15 mmol, 80% in H_2O) was added. The solution was heated at 50 ºC for 1 h, at which point a white precipitate formed and the reaction was cooled to room temperature. The precipitate was filtered, washed with water, and recrystallized from ethanol to yield the thiosemicarbazide **2-34** as a white crystalline solid (0.122 g, 62% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ

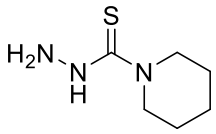
8.91 (s, 1H)

4.72 (s, 2H)

3.73 – 3.65 (m, 4H)

1.63 – 1.52 (m, 2H)

1.49 – 1.39 (m, 4H).



2-34

^{13}C NMR (125 MHz, DMSO-d_6) δ 181.99, 48.46, 25.25, 24.03.

DART-MS: *m/z* calcd for $C_6H_{14}N_3S$ $(M+H)^+$ 160.09029, found 160.09035.

***N*'-((3-Aminopyridin-2-yl)methylene)piperidine-1-carbothiohydrazide (2-43).** The aldehyde **2-14** (126.7 mg, 0.57 mmol) and the thiosemicarbazide **2-34** (90.6 mg, 0.57 mmol) were dissolved in ethanol (1 mL) and the mixture was refluxed for 4 h. The precipitate was collected by filtration and washed with cold ethanol. The crude solid was dissolved in chloroform (2 mL) and trifluoroacetic acid (0.26 mL, 3.42 mmol) was added dropwise. The mixture was stirred for 1 h and sat. $NaHCO_3$ was added to reach pH 9. A yellow precipitate formed immediately and the mixture was stirred for 1 h. The solid was collected by filtration, washed with water, and recrystallized from ethanol to yield the thiosemicarbazide **2-43** as a yellow solid (0.098 g, 66% yield).

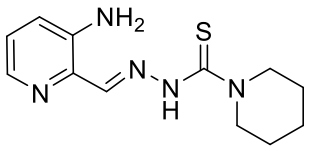
^{1}H NMR (500 MHz, Acetonitrile-d_3) δ

9.65 (s, 1H)

8.33 (s, 1H)

7.90 (dd, *J* = 4.3, 1.5 Hz, 1H)

7.09 (ddd, *J* = 8.3, 1.5, 0.5 Hz, 1H)



2-43

7.04 (dd, *J* = 8.3, 4.3 Hz, 1H)

6.50 (s, 2H)

3.92 – 3.88 (m, 4H)

1.72 – 1.67 (m, 2H)

1.66 – 1.60 (m, 4H).

^{13}C NMR (125 MHz, Acetonitrile-d_3) δ 180.76, 149.70, 144.95, 138.92, 135.74, 125.40, 123.62, 51.34, 26.89, 25.42.

DART-MS: *m/z* calcd for $C_{12}H_{18}N_5S$ $(M+H)^+$ 264.12774, found 264.12488.

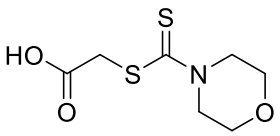
2-((Morpholine-4-carbonothioyl)thio)acetic acid (2-27). To a solution of morpholine (200 mg, 2.30 mmol) and sodium hydroxide (96.4 mg, 2.41 mmol) in ethanol (2 mL) was added dropwise carbon disufide (0.138 mL, 2.29 mmol). The mixture was stirred for 4 h then cooled to 0º C. Sodium chloroacetate (0.267 g, 2.29 mmol) in water (1 mL) was added. The mixture was allowed to warm to room temperature and stirred for 12 h. The solution was acidified to pH 2 with HCl (12 M in H_2O), and a white precipitate formed which was filtered and washed with 1.2 M HCl and water. The product was recrystallized from ethanol to yield the carbamodithioate **2-27** as a white crystalline solid (0.401 g, 79% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

12.75 (s, 1H)

4.20 (s, 2H)

4.14 (s, 2H)



2-27

3.94 (s, 2H)

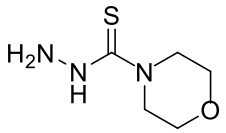
3.68 (t, J = 4.9 Hz, 4H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 195.39, 169.66, 66.04, 51.83, 50.70, 38.87.

DART-MS: *m/z* calcd for $C_7H_{12}NO_3S_2$ $(M+H)^+$ 222.02531, found 222.02470.

Morpholine-4-carbothiohydrazide (2-35). The carbamodithioate **2-27** (0.300 g, 1.36 mmol) was suspended in H_2O (2 mL), and hydrazine hydrate (0.329 mL, 6.78 mmol, 80% in H_2O) was added. The solution was heated at 50 ºC for 1 h, at which point a white precipitate formed and the reaction was cooled to room temperature. The precipitate was filtered, washed with water, and recrystallized from ethanol to yield the thiosemicarbazide **2-35** as a white crystalline solid (0.113 g, 72% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ



9.13 (s, 1H)

4.34 (s, 2H) 2-35

3.68 (dd, J = 5.7, 4.0 Hz, 4H)

3.56 (dd, J = 5.7, 4.0 Hz, 4H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 182.93, 65.69, 47.75.

DART-MS: *m/z* calcd for $C_5H_{12}N_3OS$ $(M+H)^+$ 162.06956, found 162.06923.

***N*'-((3-Aminopyridin-2-yl)methylene)morpholine-4-carbothiohydrazide (2-44).** The aldehyde **2-14** (96.4 mg, 0.434 mmol) and the thiosemicarbazide **2-35** (70.0 mg, 0.434 mmol) were dissolved in ethanol (1 mL) and the mixture was refluxed for 4 h. The precipitate was collected by filtration and washed with cold ethanol. The crude solid was dissolved in chloroform (2 mL) and trifluoroacetic acid (0.2 mL, 2.60 mmol) was added dropwise. The mixture was stirred for 1 h and sat. $NaHCO_3$ was added to reach pH 9. A yellow precipitate formed immediately and the mixture was stirred for 1 h. The solid was collected by filtration, washed with water, and recrystallized from ethanol to yield the thiosemicarbazide **2-44** as a yellow solid (0.069 g, 60% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

11.37 (s, 1H)

8.49 (s, 1H)

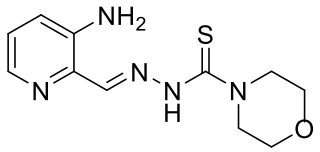
7.83 (dd, J = 4.2, 1.6 Hz, 1H)

7.16 (s, 2H)

7.11 – 7.04 (m, 2H)

3.94 – 3.89 (m, 4H)

3.69 – 3.63 (m, 4H).



2-44

^{13}C NMR (125 MHz, DMSO-d_6) δ 179.90, 149.10, 143.77, 136.70, 133.74, 124.02, 121.99, 65.78, 48.84. DART-MS: *m/z* calcd for $C_{11}H_{16}N_5OS$ $(M+H)^+$ 266.10701, found 266.10706.

Allyl 4-formyl-1-methylisoquinoline-2(1H)-carboxylate (2-58). To a solution of isoquinoline (0.100 g, 0.774 mmol) in THF (1 mL) at 0 ºC was added methylmagnesium bromide (0.387 mL, 1.16 mmol, 3.0 M in THF). Allyl chloroformate (0.103 g, 0.852 mmol) was added dropwise and the mixture was stirred for 3 h. Saturated aqueous NH_4Cl (1 mL) was added and the aqueous phase was extracted with diethyl ether (2 x1 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude product was used in the next reaction without further purification. *N*,*N*-Dimethylformamide (DMF, 0.475 mL, 6.106 mmol) was dissolved in dichloromethane (1 mL) at 0 ºC. Phosphoryl chloride (0.279 mL, 3.053 mmol) was added dropwise and the mixture was stirred for 20 min. The crude product of the previous reaction was dissolved in dichloromethane (0.5 mL) and the solution was added to the reaction mixture, which was allowed to warm to room temperature and stirred overnight. Saturated aqueous NH_4Cl (5 mL) and the aqueous phase was extracted with dichloromethane (3 x 3 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (5% ethyl acetate/hexanes) to give the aldehyde **2-58** (0.117 g, 59% yield over 2 steps).

^{1}H NMR (300 MHz, DMSO-d_6) δ

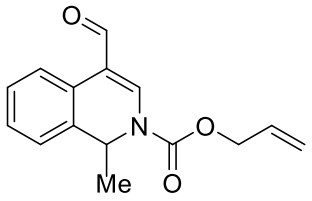
δ 9.55 (s, 1H)

8.47 (dd, *J* = 7.3, 1.9 Hz, 1H)

7.70 (appbr s, 1H)

7.25 –7.34 (m, 2H)

7.11 (dd, *J* = 7.2, 1.9 Hz, 1H)



2-58

6.04 (ddt, J = 16.3, 10.4, 5.9 Hz, 1H)

5.50 – 5.32 (m, 3H)

4.91 – 4.77 (m, 2H)

1.37 (d, J = 6.6 Hz, 3H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 189.28, 151.88, 144.83, 133.35, 131.32, 128.36, 127.90, 125.81, 125.56, 124.83, 119.68, 118.18, 68.13, 53.37, 22.89.

DART-MS: *m/z* calcd for $C_{15}H_{16}NO_3$ $(M+H)^+$ 258.11247, found 258.11193.

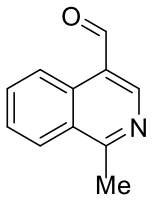
1-Methylisoquinoline-4-carbaldehyde (2-54). To a degassed solution of aldehyde **2-58** (0.055 g, 0.214 mmol) in dichloromethane (1 mL) was added tetrakis(triphenylphosphine)palladium(0) (0.5 mg, 4.0 x 10^{-4} mmol) and morpholine (0.022 mL, 0.256 mmol). The mixture was stirred at room temperature for 1 h then diluted with dichloromethane (1 mL) and cooled to 0 ºC. DDQ (0.0533 g, 0.235 mmol) was added portionwise and the mixture was stirred for 30 min at 0 ºC. The reaction mixture was slowly poured into a solution of saturated aqueous $NaHCO_3$ and extracted with dichloromethane (3 x 3 mL). The combined extracts were washed with brine (3 mL) and dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (10% ethyl acetate/hexanes) to give the isoquinoline **2-54** (0.028 g, 77%).

^{1}H NMR (300 MHz, DMSO-d_6) δ

10.31 (s, 1H)

9.24 (dd, J = 8.4, 1.0 Hz, 1H)

8.78 (s, 1H)



8.28 – 8.19 (m, 1H)

7.88 (ddd, *J* = 8.4, 7.0, 1.3 Hz, 1H)

7.72 (ddd, *J* = 8.3, 7.0, 1.3 Hz, 1H) 2-54

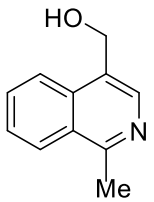
3.06 (s, 3H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 192.71, 165.76, 152.11, 133.07, 132.50, 128.28, 127.13, 126.12, 125.10, 124.03, 23.42.

DART-MS: *m/z* calcd for $C_{11}H_{10}NO$ $(M+H)^+$ 172.07569, found 172.07664.

4-(Hydroxymethyl)-1-methylisoquinoline (2-59). To the aldehyde **2-54** (0.100 g, 0.584 mmol) in methanol (10 mL) and dichloromethane (5 mL) was added cerium trichloride (0.216 g, 0.584 mmol). The mixture was cooled to 0 ºC and sodium borohydride (0.022 g, 0.584 mmol) was slowly added. The reaction was stirred for 30 min then carefully quenched with water and extracted with dichloromethane (3 x 8 mL). The combined extracts were dried over $MgSO_4$, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (25% ethyl acetate/hexanes) to give the alcohol **2-59** (0.089 g, 89%).

^{1}H NMR (300 MHz, DMSO-d_6) δ



8.51 – 8.42 (m, 2H)

8.37 (dt, *J* = 8.6, 1.4 Hz, 1H)

8.09 – 7.97 (m, 1H) 2-59

7.90 (ddd, *J* = 8.2, 6.9, 1.3 Hz, 1H)

5.32 (s, 2H)

4.55 (s, 1H)

3.13 (s, 3H).

^{13}C NMR (75MHz, DMSO-d_6) δ 158.98, 140.35, 134.41, 130.33, 128.58, 127.14, 126.96, 126.04, 123.97, 60.92, 22.10.

DART-MS: *m/z* calcd for $C_{11}H_{12}NO$ $(M+H)^+$ 174.09134, found 174.09002.

4-(Azidomethyl)-1-methylisoquinoline (2-53). To a solution of the alcohol **2-59** (0.230 g, 1.33 mmol) in THF (10 mL) was added diphenylphosphoryl azide (0.438 g, 1.59 mmol). The mixture was cooled to 0 ºC and DBU (0.222 g, 1.461 mmol) was added. The reaction was allowed to warm to room temperature and stirred overnight, then diluted with ethyl acetate (10 mL) and washed with water (20 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography on silica gel (25% ethyl acetate/hexanes) to give the azide **2-53** (0.137 g, 52%).

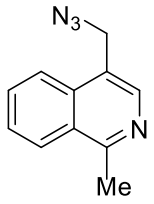
^{1}H NMR (500 MHz, DMSO-d_6) δ

8.35 (s, 1H)

8.19 (dd, *J* = 8.5, 1.0 Hz, 1H)

8.02 (dd, *J* = 8.4, 1.0 Hz, 1H)

7.79 (ddd, *J* = 8.3, 6.9, 1.2 Hz, 1H)



2-53

7.67 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H)

4.69 (s, 2H)

2.99 (s, 3H).

^{13}C NMR (100MHz, DMSO-d_6) δ 160.40, 141.89, 134.25, 130.91, 127.48, 127.41, 126.47, 123.47, 123.18, 50.49, 22.50.

DART-MS: *m/z* calcd for $C_{11}H_{11}N_4$ $(M+H)^+$ 199.09784, found 199.09689.

4-(Chloromethyl)-1-methylisoquinoline (2-60). To a solution of the alcohol **2-59** (0.300 g, 1.73 mmol) in dichloromethane (7 mL) was added thionyl chloride (2.0 mL, 29.1 mmol). The mixture was stirred for 4 h and concentrated *in vacuo*. To the residue was added saturated aqueous $NaHCO_3$ (5 mL) and the aqueous phase was extracted with dichloromethane (3 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated to produce the chloride **2-60** (0.305 g, 92%).

^{1}H NMR (300 MHz, DMSO-d_6) δ

8.41 (s, 1H)

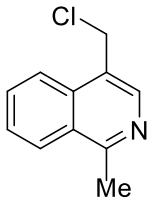
8.18 (dt, *J* = 8.4, 1.0 Hz, 2H)

8.13 (dt, *J* = 8.4, 1.0 Hz, 2H)

7.81 (ddd, *J* = 8.4, 6.9, 1.3 Hz, 1H)

7.66 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H)

4.99 (s, 2H)



2-60

2.98 (s, 3H).

^{13}C NMR (75 MHz, DMSO-d_6) δ 160.57, 142.27, 133.88, 130.71, 127.36, 126.45, 125.30, 123.54, 120.00, 41.96, 22.65.

DART-MS: *m/z* calcd for $C_{11}H_{11}ClN$ $(M+H)^+$ 192.05745, found 199.05698.

4-(Chloromethyl)-1-methyl-5-nitroisoquinoline (2-61). Sulfuric acid (1 mL) was cooled to 0 ºC and the chloride **2-60** (0.269 g, 1.04 mmol) was added slowly. Potassium nitrate (0.156 g, 1.55 mmol) was then added. The reaction mixture was gradually heated at 60 ºC and stirred for 2 h. The mixture was cooled to room temperature and poured over ice. The solution was neutralized to pH 7 using potassium carbonate, and the resulting precipitate was collected by filtration, washed with water, and recrystallized from ethanol to give the isoquinoline **2-61** (0.239 mg, 72%).

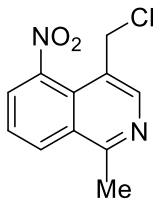
^{1}H NMR (400 MHz, DMSO-d_6) δ

8.56 (s, 1H)

8.48 (dd, *J* = 8.4, 1.3 Hz, 1H)

8.15 (dd, *J* = 7.6, 1.3 Hz, 1H)

7.74 (dd, *J* = 8.4, 7.6 Hz, 1H)



2-61

4.93 (s, 2H)

3.06 (s, 3H).

^{13}C NMR (100 MHz, DMSO-d_6) δ 161.04, 146.74, 135.04, 131.51, 128.04, 126.12, 125.50, 123.28, 122.46, 44.11, 23.58.

DART-MS: *m/z* calcd for $C_{11}H_{10}ClN_2O_2$ $(M+H)^+$ 237.04253, found 199.04214.

Isoquinoline-1-carboxaldehyde (2-66a). To a solution of 1-methylisoquinoline (1.0 g, 6.98 mmol) in 1,4-dioxane (10 mL) was added selenium dioxide (0.930 g, 8.38 mmol) and the mixture was refluxed for 4 h. The mixture was filtered, then concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (25% dichloromethane/hexanes) to give the aldehyde **2-66a** as a taupe powder (0.840 g, 69% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

10.28 (s, 1H)

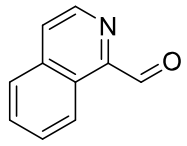
9.15 (ddd, *J* = 7.7, 1.9, 0.8 Hz, 1H)

8.82 (d, *J* = 5.5 Hz, 1H)

8.21 (dd, *J* = 5.6, 0.9 Hz, 1H)

8.17–8.12 (m, 1H)

7.93–7.84 (m, 2H).



2-66a

^{13}C NMR (125 MHz, DMSOd_6) δ 195.64, 149.38, 142.47, 136.49, 131.00, 130.30, 127.45, 125.77, 125.41, 124.73.

DART-MS: *m/z* calcd for $C_{10}H_8NO$ $(M+H)^+$ 158.06004, found 158.05977.

(*E*)-2-(Isoquinolin-1-ylmethylene)hydrazine-1-carbothioamide (2-7). To a solution of the aldehyde **2-66a** (400 mg, 2.55 mmol) in ethanol (4 mL) was added thiosemicarbazide (232 mg, 2.55 mmol) and HCl (2.12 mL, 2.55 mmol, 1.2 M in H_2O). The mixture was stirred and refluxed for 1.5 h then cooled to 22 °C. The hydrochloride salt that formed was then neutralized with saturated aqueous $NaHCO_3$ solution (4 mL). The precipitate of the desired compound was

collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline **2-7** as a pale-yellow solid (482 mg, 82% yield).

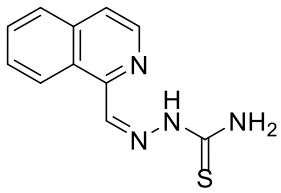
^{1}H NMR (500 MHz, DMSO-d_6) δ

11.74 (s, 1H)

9.19 (d, J = 8.5 Hz, 1H)

8.60 – 8.54 (m, 2H)

8.49 (s, 1H)



2-7

8.02 (d, J = 8.1 Hz, 1H)

7.86 (d, J = 5.6 Hz, 1H)

7.84 – 7.78 (m, 2H)

7.75 (ddd, J = 8.3, 6.8, 1.4 Hz, 1H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 178.41, 150.78, 145.99, 142.13, 136.24, 130.47, 129.08, 127.22, 126.94, 125.58, 121.77.

DART-MS: *m/z* calcd for $C_{11}H_{11}N_4S$ $(M+H)^+$ 231.06989, found 231.06938.

(*E*)-2-(Isoquinolin-1-ylmethylene)-*N*-methylhydrazine-1-carbothioamide (2-72). To a solution of the aldehyde **2-66a** (0.060 g, 0.382 mmol) in ethanol (3 mL) was added 4-methyl-3-thiosemicarbazide (0.040g, 0.382 mmol) and HCl (0.318 mL, 12 M in H_2O). The mixture was refluxed for 4 h. The solid that formed was collected by filtration, washed with water, and

recrystallized from ethanol to yield the isoquinoline **2-72** as a yellow powder (0.058 g, 62% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

11.78 (br s, 1H)

9.11 (br s, 1H)

8.61 (s, 1H)

8.56 (d, J = 5.6 Hz, 1H)

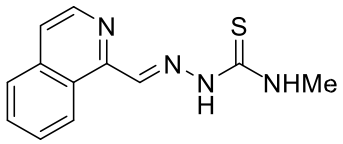
8.31 (br s, 1H)

8.02 (d, J = 8.1 Hz, 1H)

7.89–7.79 (m, 2H)

7.76 (t, J = 7.7 Hz, 1H)

3.07 (s, 3H).



2-72

^{13}C NMR (125 MHz, DMSO-d_6) δ 178.36, 151.06, 144.76, 142.15, 136.25, 130.42, 128.86, 127.21, 126.82, 125.64, 121.48, 31.34.

DART-MS: m/z calcd for $C_{12}H_{13}N_4S$ $(M+H)^+$ 245.08554, found 245.08505.

(*E*)-2-(Isoquinolin-1-ylmethylene)-*N*,*N*-dimethylhydrazine-1-carbothioamide and (*Z*)-2(Isoquinolin-1-ylmethylene)-*N*,*N*-dimethylhydrazine-1-carbothioamide (2-77). To a solution of the aldehyde **2-66a** (0.060 g, 0.382 mmol) in ethanol (3 mL) was added 4,4-dimethyl-3-thiosemicarbazide (0.046g, 0.382 mmol) and HCl (0.318 mL, 12 M in H_2O). The mixture was refluxed for 4 h. The solid that formed was collected by filtration, washed with water, and recrystallized from ethanol to yield the isoquinoline **2-77** as a yellow powder (0.056 g, 57% yield) (mixture of E and Z isomers).

^{1}H NMR (500 MHz, DMSO-*d6*) δ

15.99 (s, 0.33H)

11.26 (br s, 1H)

9.77 (dd, *J* = 8.8, 5.1 Hz, 1H)

8.81 (d, *J* = 8.6 Hz, 0.33H)

8.70 (d, *J* = 1.7 Hz, 1H)

8.69 (d, *J* = 1.2 Hz, 0.33H)

8.63 (s, 0.33H)

8.55 (d, *J* = 5.5 Hz, 1H)

8.12 (d, *J* = 8.2 Hz, 0.33H)

8.01–7.96 (m, 1.33H)

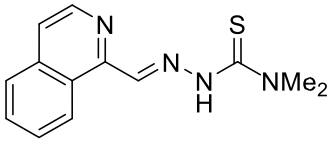
7.92 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 0.33H)

7.88–7.76 (m, 2.33H)

7.72 (ddd, *J* = 8.4, 6.8, 1.4 Hz, 1H)

3.43 (s, 1.98H)

3.35 (s, 6H).



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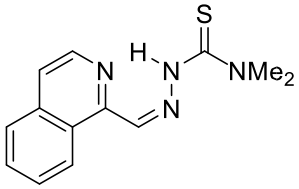


2-77

^{13}C NMR (125 MHz, DMSO-d_6) δ 180.88, 180.81, 151.94, 150.58, 147.81, 142.50, 140.45, 136.86, 136.82, 131.80, 130.77, 129.48, 129.16, 128.21, 128.17, 127.68, 126.83, 125.87, 124.60, 122.53, 121.93, 42.08.

DART-MS: m/z calcd for $C_{13}H_{15}N_4S$ $(M+H)^+$ 259.10119, found 259.10080.

1-Methyl-5-nitroisoquinoline (2-65a.i). To a solution of 1-methylisoquinoline (28.80 g, 201.2 mmol) in sulfuric acid (92.4 mL) at 0 ˚C was added KNO_3 (20.4 g, 201.2 mmol) in sulfuric acid (78.0 mL). The mixture was heated at 60 ˚C for 2 h and then poured slowly over crushed ice. The solution was made alkaline with NH_4OH; the resulting tan precipitate was filtered, washed with water, and dried to afford the isoquinoline **2-65a.i** as a tan solid (20.00 g, 53% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

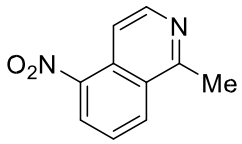
8.61 (d, *J* = 6.2 Hz, 1H)

8.47–8.50 (m, 2H)

8.28 (d, *J* = 6.3 Hz, 1H)

7.71 (t, *J* = 8.1 Hz, 1H)

3.05 (s, 3H).



2-65a.i

^{13}C NMR (125 MHz, $CDCl_3$) δ 159.53, 145.38, 132.53, 128.65, 128.23, 127.79, 125.58, 114.26, 23.38.

DART-MS: *m/z* calcd for $C_{10}H_9N_2O_2$ $(M+H)^+$ 189.06585, found 189.06544.

1-Methylisoquinolin-5-amine (2-65a.ii). To a solution of the isoquinoline **2-65a.i** (20.00 g, 106.28 mmol) and iron powder (44.40 g, 795.05 mmol) in methanol (530 mL) was added concentrated HCl (1 mL, 12 M in H_2O). The mixture was refluxed for 2 h and then a solution of sodium hydroxide (6 mL, 2 M in H_2O) was added. The mixture was filtered, then concentrated *in vacuo*, and resuspended in EtOAc (200 mL) and water (200 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (3 × 200 mL). The organic layers

were combined and dried over Na_2SO_4, filtered, and then concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel (gradient, 10–30% ethyl acetate/hexanes). The amine **2-65a.ii** was obtained as a brown solid (15.0 g, 90% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

8.36 (d, *J* = 6.1 Hz, 1H)

7.55 (dt, *J* = 8.4, 1.0 Hz, 1H)

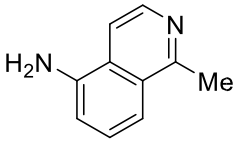
7.45 (d, *J* = 5.7 Hz, 1H)

7.39 (dd, *J* = 8.5, 7.4 Hz, 1H)

6.95 (dd, *J* = 7.5, 0.9 Hz, 1H)

4.18 (br s, 2H)

2.93 (s, 3H).



2-65a.ii

^{13}C NMR (125 MHz, $CDCl_3$) δ 165.39, 159.15, 141.94, 128.35, 127.51, 126.16, 116.19, 113.09, 112.73, 23.06.

DART-MS: *m/z* calcd for $C_{10}H_{11}N_2$ $(M+H)^+$ 159.09167, found 159.09136.

***tert*-Butyl (*tert*-butoxycarbonyl)(1-methylisoquinolin-5-yl)carbamate (2-65a.iii).** To a solution of the amine **2-65a.ii** (360.0 mg, 2.28 mmol) in THF (10 mL) was added Boc_2O (1.68 g, 6.83 mmol), DMAP (28.0 mg, 0.23 mmol), and triethylamine (0.69 g, 3.65 mmol) and the mixture was stirred at 22 °C overnight. The reaction was quenched with water (10 mL) and the organic layers were separated. The aqueous layer was extracted with ethyl acetate (3 × 10 mL).

The organic layers were combined and dried over Na_2SO_4, filtered, and then concentrated *in vacuo*. The crude residue was purified by flash column chromatography (gradient, 10–30% EtOAc/hexanes). The dicarbamate **2-65a.iii** was obtained as a brown solid (420.0 mg, 51% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

8.43 (d, *J* = 6.0 Hz, 1H)

8.11 (d, *J* = 8.5 Hz, 1H)

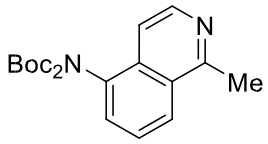
7.58 (t, *J* = 7.9 Hz, 1H)

7.51 (dd, *J* = 7.3, 1.1 Hz, 1H)

7.46 (d, *J* = 5.9 Hz, 1H)

2.99 (s, 3H)

1.31 (s, 18H).



2-65a.iii

^{13}C NMR (125 MHz, $CDCl_3$) δ 159.16, 151.59, 142.73, 135.74, 133.82, 129.64, 128.20, 126.55, 125.86, 113.74, 83.19, 27.90, 22.90.

DART-MS: *m/z* calcd for $C_{20}H_{27}N_2O_4$ $(M+H)^+$ 359.19653, found 359.19540.

***tert*-Butyl (1-methylisoquinolin-5-yl)carbamate (2-65a.iv).** To a solution of the amine **2-65a.ii** (10.00 g, 63.21 mmol) in THF (250 mL) was added Boc_2O (34.38 g, 158.0 mmol), DMAP (772.2 mg, 6.32 mmol), and triethylamine (15.96 g, 158.0 mmol) and the mixture was stirred at 22 °C overnight. After completion of the reaction as judged by TLC, $NaHCO_3$ (15.93 g, 189.6 mmol)

and methanol (100 mL) were added to the reaction mixture and it was refluxed overnight. After completion of the reaction (monitored by TLC), the mixture was concentrated *in vacuo* and then resuspended in ethyl acetate (200 mL) and water (200 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 200 mL). The organic layers were combined and dried over Na_2SO_4, filtered, and then concentrated *in vacuo*. The crude residue was purified by flash column chromatography (gradient, 10–30% ethyl acetate/hexanes). The carbamate **2-65a.iv** was obtained as a brown oil (4.73 g, 29% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

8.37 (d, *J* = 6.1 Hz, 1H)

7.56 (dt, *J* = 8.3, 1.0 Hz, 1H)

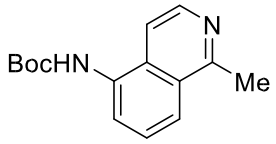
7.46 (d, *J* = 6.7 Hz, 1H)

7.40 (dd, *J* = 8.5, 7.4 Hz, 1H)

6.95 (dd, *J* = 7.5, 0.9 Hz, 1H)

2.94 (s, 4H)

1.56 (s, 9H).



2-65a.iv

^{13}C NMR (125 MHz, $CDCl_3$) δ 165.39, 159.15, 141.94, 128.35, 127.51, 126.16, 116.19, 113.09, 112.73, 76.91, 29.86, 23.06, one low-field carbon was either not observed or is overlapping with another lowfield carbon.

DART-MS: *m/z* calcd for $C_{15}H_{19}N_2O_2$ $(M+H)^+$ 259.14410, found 259.14349.

***tert*-Butyl methyl(1-methylisoquinolin-5-yl)carbamate (2-65a.v).** To a solution of the carbamate **2-65a.iv** (1.99 g, 7.68 mmol) in THF (50 mL) was added NaH 60% in mineral oil (399.6 mg, 9.99 mmol). After effervescence ceased, the resulting solution was refluxed for 30 min. To the reaction mixture was then added MeI (622 µL, 9.99 mmol) in THF (2 mL) and the solution was subsequently refluxed overnight. The mixture was concentrated *in vacuo* and the crude residue was purified by flash column chromatography (gradient, 10–30% ethyl acetate/hexanes). The isoquinoline **2-65a.v** was obtained as an amber oil (4.73 g, 29% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

8.42 (d, *J* = 6.0 Hz, 1H)

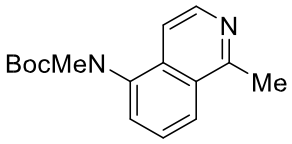
8.08 (d, *J* = 8.4 Hz, 1H)

7.46–7.61 (m, 3H)

3.31 (s, 3H)

3.01 (s, 3H)

1.23 (s, 9H).



2-65a.v

^{13}C NMR (125 MHz, $CDCl_3$) δ 159.01, 155.21, 140.21, 133.54, 128.35, 126.95, 124.96, 121.52, 114.65, 76.15, 29.71, 28.06, 22.51, one low-field carbon was either not observed or is overlapping with another low-field carbon.

DART-MS: *m/z* calcd for $C_{16}H_{21}N_2O_2$ $(M+H)^+$ 273.15975, found 273.15891.

***tert*-Butyl (1-formylisoquinolin-5-yl)(methyl)carbamate (2-68a).** To a solution of the isoquinoline **2-65a.v** (1.50 g, 5.51 mmol) in 1,4-dioxane (60 mL) was added SeO_2 (1.22 g, 11.0

mmol). The mixture was stirred at 60 °C overnight then cooled to 22 °C. The mixture was concentrated *in vacuo* and the crude residue was purified by flash column chromatography on silica gel (gradient, 5–25% ethyl acetate/hexanes). The aldehyde **2-68a** was obtained as a white solid (711.9 mg, 45% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

10.39 (s, 1H)

9.28 (d, *J* = 8.6 Hz, 1H)

8.80 (d, *J* = 5.7 Hz, 1H)

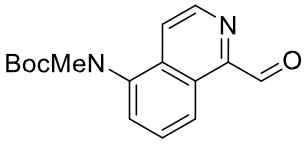
7.88 (d, *J* = 5.1 Hz, 1H)

7.74 (d, *J* = 8.0 Hz, 1H)

7.61 (m, 1H)

3.33 (s, 3H)

1.22 (s, 9H).



2-68a

^{13}C NMR (125 MHz, $CDCl_3$) δ 195.44, 155.05, 150.18, 142.85, 139.95, 134.55, 129.90, 128.96, 127.08, 124.99, 120.65, 80.72, 37.85, 28.08.

DART-MS: *m/z* calcd for $C_{16}H_{19}N_2O_3$ $(M+H)^+$ 287.13902, found 287.13812.

***tert*-Butyl (*tert*-butoxycarbonyl)(1-formylisoquinolin-5-yl)carbamate (2-67).** To a solution of the isoquinoline **2-65a.iii** (200.0 mg, 0.558 mmol) in 1,4-dioxane (5.5 mL) was added SeO_2 (123.8 mg, 1.12 mmol). The mixture was stirred at 60 °C overnight then cooled to 22 °C. The

mixture was concentrated *in vacuo* and the crude residue was purified by flash column chromatography on silica gel (gradient, 5–25% ethyl acetate/hexanes). The aldehyde **2-67** was obtained as a white solid (63.2 mg, 30% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

10.40 (s, 1H)

9.34 (dt, *J* = 8.7, 1.0 Hz, 1H)

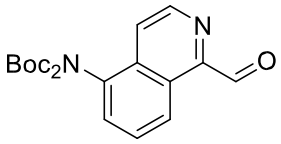
8.81 (d, *J* = 5.7 Hz, 1H)

7.89 (dd, *J* = 5.7, 1.0 Hz, 1H)

7.76 (dd, *J* = 8.7, 7.4 Hz, 1H)

7.61 (dd, *J* = 7.3, 1.1 Hz, 1H)

1.32 (s, 18H).



2-67

^{13}C NMR (125 MHz, $CDCl_3$) δ 195.59, 151.36, 150.23, 143.31, 134.94, 130.49, 129.74, 126.88, 126.00, 119.90, 83.60, 27.91, two low-field carbons were either not observed or are overlapping with another low-field carbon.

DART-MS: *m/z* calcd for $C_{20}H_{25}N_2O_5$ $(M+H)^+$ 373.17580, found 373.17496.

(*E*)-2-((5-(Methylamino)isoquinolin-1-yl)methylene)hydrazine-1-carbothioamide (2-49). To a solution of the aldehyde **2-68a** (100.0 mg, 0.3492 mmol) in ethanol (1.75 mL) was added thiosemicarbazide (31.8 mg, 0.3492 mmol) and HCl (350 µL, 6 M in H_2O). The mixture was stirred and refluxed for 1.5 h and then cooled to 22 °C. The hydrochloride salt that formed was

neutralized with 1.4 mL of a saturated aqueous $NaHCO_3$ solution. The precipitate of the desired compound was collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline **2-49** as a black solid (622.4 mg, 97% yield).

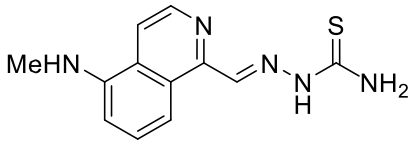
^{1}H NMR (500 MHz, DMSOd_6) δ

12.32 (s, 1H)

9.07 (br s, 1H)

8.92 (s, 1H)

8.90 (s, 1H)



2-49

8.57 (d, *J* = 6.6 Hz, 1H)

8.52 (d, *J* = 6.7 Hz, 1H)

7.85 (t, *J* = 8.2 Hz, 1H)

7.56 (d, *J* = 8.3 Hz, 1H)

7.30 (br s, 1H)

7.01 (d, *J* = 8.0 Hz, 1H)

2.91 (s, 3H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 179.40, 146.25, 146.00, 133.09, 130.09, 128.72, 126.75, 119.38, 111.17, 110.66, 30.39.

DART-MS: *m/z* calcd for $C_{12}H_{14}N_5S$ $(M+H)^+$ 260.09644, found 260.09501.

(*E*)-*N*-Methyl-2-((5-(methylamino)isoquinolin-1-yl)methylene)hydrazine-1-carbothioamide and (*Z*)-*N*-Methyl-2-((5-(methylamino)isoquinolin-1-yl)methylene)hydrazine-1-carbothioamide (2-75). To a solution of the aldehyde **2-68a** (51.4 mg, 0.18 mmol) in ethanol (0.88 mL) was added 4-methyl-3-thiosemicarbazide (18.9 mg, 0.18 mmol) and HCl (0.18 mL, 6 M in H_2O). The mixture was stirred and refluxed for 1.5 h then cooled to 22 °C. The hydrochloride salt that formed was neutralized with saturated aqueous $NaHCO_3$ solution (0.88 mL). The precipitate of the desired compound was collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline **2-75** as a black solid (49.0 mg, 94% yield) (mixture of *E* and *Z* isomers).

^{1}H NMR (500 MHz, DMSO-d_6) δ

14.74 (s, 0.15H)

12.22 (s, 1H)

9.39 (br s, 1H)

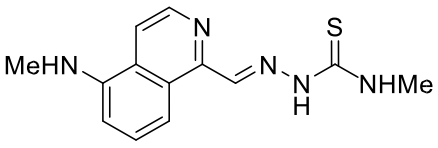
8.93 (q, *J* = 4.7 Hz, 0.15H)

8.78 (s, 1H)

8.54 (d, *J* = 5.9 Hz, 0.15H)

8.50 (d, *J* = 6.5 Hz, 1H)

8.38 (s, 1H)



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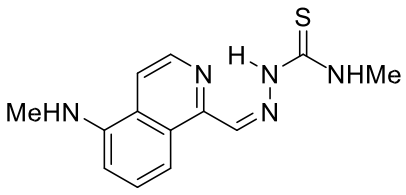


8.17 (s, 0.15H)

8.12 (d, *J* = 5.9 Hz, 0.15H) 2-75

7.82 (d, *J* = 8.6 Hz, 0.15H)

7.76 (t, *J* = 8.1 Hz, 1H)

7.69 (s, 1H)

7.56 (t, *J* = 8.1 Hz, 0.15H)

7.08 (br s, 1H)

6.92 (d, *J* = 7.7 Hz, 1.15H)

6.72 (d, *J* = 7.8 Hz, 0.15H)

3.07 (d, *J* = 4.6 Hz, 3H)

3.02 (d, *J* = 4.6 Hz, 0.45 H)

2.88 (s, 3H)

2.86 (s, 0.45H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 178.84, 178.38, 150.11, 147.37, 145.83, 145.56, 138.94, 132.45, 130.58, 129.19, 128.23, 128.22, 126.87, 126.80, 118.47, 117.04, 111.57, 110.13, 109.63, 106.57, 31.59, 31.42, 30.42.

DARTMS: *m/z* calcd for $C_{13}H_{16}N_5S$ $(M+H)^+$ 274.11209, found 274.11104.

(*E*)-2-((5-Aminoisoquinolin-1-yl)methylene)hydrazine-1-carbothioamide (2-71). To a solution of the aldehyde **2-67** (30.0 mg, 0.081 mmol) in ethanol (0.39 mL) was added thiosemicarbazide (7.3 mg, 0.802 mmol) and HCl (80.6 µL, 6 M in H_2O). The mixture was stirred and refluxed for 1.5 h then cooled to 22 °C. The hydrochloride salt that formed was neutralized

with saturated aqueous $NaHCO_3$ solution (0.39 mL). The precipitate of the desired compound was collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline **2-71** as a green solid (19.6 mg, 99% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

11.66 (br s, 1H)

8.57 (s, 1H)

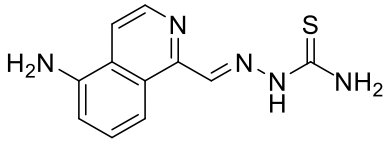
8.42 (d, J = 5.8 Hz, 1H)

8.31 (br s, 1H)

8.25 (d, J = 8.5 Hz, 1H)

7.98 (d, J = 5.8 Hz, 1H)

7.60 (br s, 1H)



2-71

7.42 (t, J = 8.1 Hz, 1H)

6.89 (d, J = 7.1 Hz, 1H)

6.02 (s, 2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 178.46, 150.36, 145.86, 144.62, 140.01, 129.74, 126.78. 125.83. 116.50, 113.12, 110.74.

DART-MS: *m/z* calcd for $C_{11}H_{11}N_5S$ $(M+H)^+$ 246.08079, found 246.08020.

(*E*)-2-((5-Aminoisoquinolin-1-yl)methylene)-*N*-methylhydrazine-1-carbothioamide and (*Z*)- 2-((5Aminoisoquinolin-1-yl)methylene)-*N*-methylhydrazine-1-carbothioamide (2-76). To a solution of the aldehyde **2-67** (27.1 mg, 0.0728 mmol) in ethanol (0.73 mL) was added 4-methyl-3-thiosemicarbazide (7.7 mg, 0.0732 mmol) and HCl (72.8 µL, 6 M in H_2O). The mixture was stirred and refluxed for 1.5 h then cooled to 22 °C. The hydrochloride salt that formed was neutralized with saturated aqueous $NaHCO_3$ solution (0.73 mL). The precipitate of the desired compound was collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline **2-76** as a yellow solid (5.1 mg, 27% yield) (mixture of *E* and *Z* isomers).

^{1}H NMR (500 MHz, DMSO-d_6) δ

14.80 (s, 0.08H)

11.66 (br s, 1H)

8.95 (d, *J* = 4.9 Hz, 0.08H)

8.62 (s, 1H)

8.52 (d, *J* = 5.9 Hz, 0.8H)

8.42 (d, *J* = 5.8 Hz, 1H)

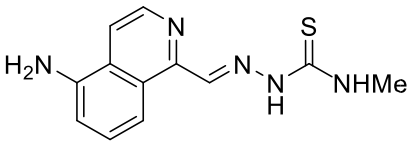
8.25 (d, *J* = 3.3 Hz, 1H)

8.18 (s, 0.08H)

8.11–8.15 (m, 1.08H)

7.99 (d, *J* = 5.9 Hz, 1H)

7.83 (d, *J* = 8.4 Hz, 0.08H)



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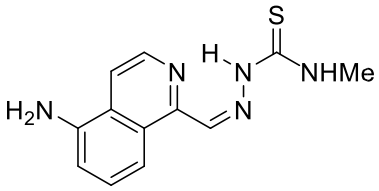


2-76

7.48 (t, *J* = 7.9 Hz, 0.08H)

7.43 (t, *J* = 8.0 Hz, 1H)

6.97 (d, *J* = 7.6 Hz, 0.08H)

6.91 (dd, *J* = 7.6, 0.9 Hz, 1H)

6.21 (s, 0.16H)

6.04 (s, 2H)

3.05– 3.07 (m, 3.24H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 178.85, 178.52, 150.83, 150.19, 145.53, 145.20, 145.10, 140.49, 138.40, 130.28, 130.10, 129.16, 128.41, 127.36, 126.27, 126.16, 117.81, 116.96, 113.24, 111.66, 111.18, 110.61, 31.74, 31.59.

DART-MS: *m/z* calcd for $C_{12}H_{14}N_5S$ $(M+H)^+$ 260.09644, found 260.09563.

4-Fluoro-1-methylisoquinoline (2-65b). To a solution of 4-fluoroisoquinoline (1.50 g, 10.19 mmol) in THF (102 mL) was added allyl chloroformate (2.17 mL, 20.38 mmol). MeMgBr (10.19 mL, 2 M in diethyl ether) was then added dropwise to the reaction mixture at 0 °C with stirring. The reaction mixture was gradually warmed to 22 °C over a period of 2 h. The mixture was quenched with saturated aqueous NH_4Cl (10 mL) and water (100 mL) was added. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 100 mL). The organic layers were combined and dried over $MgSO_4$, filtered, and then concentrated *in vacuo*. The crude residue in ethyl acetate was filtered through a silica plug, concentrated *in vacuo* and the residue was subjected to the next reaction without further purification. To a solution of the

crude residue and $Pd(PPh_3)_4$ (70.1 mg, 0.061 mmol) in dichloromethane (60 mL) at 0 °C was added morpholine (523.1 uL, 6.07 mmol). The reaction mixture was stirred and slowly warmed to 22 °C over a period of 3 h. The mixture was cooled to 0 °C and DDQ (1.38 g, 6.07 mmol) was added in portions. After the reaction mixture stirred at 0 °C for 30 min, the reaction was slowly poured into saturated $NaHCO_3$ solution (60 mL) and extracted with dichloromethane (3 × 60). The combined extracts are washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel (gradient, 5–25% ethyl acetate/hexanes). The isoquinoline **2-65b** was obtained as a brown oil (241.9 mg, 15% over three steps).

^{1}H NMR (500 MHz, $CDCl_3$) δ

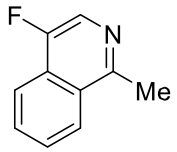
8.23 (d, *J* = 1.7 Hz, 1H)

8.06–8.09 (m, 2H)

7.73-7.76 (m, 1H)

7.64-7.67 (m, 1H)

2.90 (s, 3H).



2-65b

^{13}C NMR (125 MHz, $CDCl_3$) δ 154.48 (d, $^1J_{C\text{-}F}$ = 257.5 Hz), 154.24 (d, $^3J_{C\text{-}F}$ = 4.9 Hz), 130.14 (d, $^4J_{C\text{-}F}$ = 1.6 Hz), 128.35 (d, $^4J_{C\text{-}F}$ = 2.4 Hz), 127.83, 126.64 (d, $^2J_{C\text{-}F}$ = 15.3 Hz), 126.57 (d, $^2J_{C\text{-}F}$ = 22.3 Hz), 125.60 (d, $^4J_{C\text{-}F}$ = 2.1 Hz), 120.09 (d, $^3J_{C\text{-}F}$ = 4.5 Hz), 22.10.

^{19}F NMR (376 MHz, $CDCl_3$) δ –143.11, extraneous peak found at –139.82.

DART-MS: *m/z* calcd for $C_{10}H_9FN$ $(M+H)^+$ 162.07135, found 162.07092.

4-Fluoroisoquinoline-1-carboxaldehyde (2-66b). To a solution of the isoquinoline **2-65b** (40.0 mg, 0.248 mmol) in 1,4-dioxane (2.5 mL) was added SeO_2 (55.1 mg, 0.496 mmol). The mixture

was stirred at 60 °C overnight then cooled to 22 °C. The mixture was concentrated *in vacuo* and the crude residue was purified by flash column chromatography on silica gel (gradient, 5–25% ethyl acetate/hexanes). The aldehyde **2-66b** was obtained as a white solid (27.3 mg, 63% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

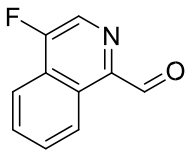
10.32 (s, 1H)

9.37–9.41 (m, 1H)

8.59 (d, J = 1.5 Hz, 1H)

8.16–8.20 (m, 1H)

7.82-7.87 (m, 2H).



2-66b

^{13}C NMR (125 MHz, DMSO-d_6) δ 194.23, 157.16 (d, $^{1}J_{C\text{-}F}$ = 270.3 Hz), 146.44 (d, $^{3}J_{C\text{-}F}$ = 5.6 Hz), 131.07 (d, $^{4}J_{C\text{-}F}$ = 2.2 Hz), 130.95 128.48 (d, $^{2}J_{C\text{-}F}$ = 24.6 Hz), 128.14 (d, $^{4}J_{C\text{-}F}$ = 4.1 Hz), 126.85 (d, $^{2}J_{C\text{-}F}$ = 14.4 Hz), 125.64 (d, $^{4}J_{C\text{-}F}$ = 1.8 Hz), 119.85 (d, $^{3}J_{C\text{-}F}$ = 4.7 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ –129.02.

DART-MS: *m/z* calcd for $C_{10}H_6FNO$ $(M+H)^+$ 176.05062, found 176.05012.

(*E*)-2-((4-Fluoroisoquinolin-1-yl)methylene)hydrazine-1-carbothioamide (2-69). To a solution of the aldehyde 2-66b (6.0 mg, 0.0343 mmol) in ethanol (0.5 mL) was added thiosemicarbazide (3.3 mg, 0.0343 mmol) and HCl (34 µL, 0.206 mmol, 6 M in H_2O). The mixture was stirred and refluxed for 1.5 h then cooled to 22 °C. The hydrochloride salt that formed was then neutralized with saturated aqueous $NaHCO_3$ solution (0.5 mL). The precipitate of the desired compound was collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline 2-69 as a pale-yellow solid (3.0 mg, 35% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

11.70 (s, 1H)

9.28 (d, J = 8.5 Hz, 1H)

8.56 (d, J = 1.5 Hz, 1H)

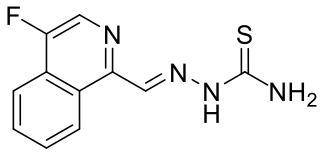
8.53 (s, 1H)

8.48 (s, 1H)

8.13 (d, J = 8.2 Hz, 1H)

7.94 (ddd, J = 8.2, 7.0, 0.9 Hz, 1H)

7.85 (m, 2H).



2-69

^{13}C NMR (125 MHz, DMSOd_6) δ 178.84, 154.73 (d, $^1J_{C\text{-}F}$ = 262.2 Hz), 148.03 (d, $^3J_{C\text{-}F}$ = 5.2 Hz), 145.84, 131.75, 130.69, 128.10 (d, $^2J_{C\text{-}F}$ = 23.3 Hz), 127.75, 127.35 (d, $^4J_{C\text{-}F}$ = 3.0 Hz), 126.51 (d, $^2J_{C\text{-}F}$ = 14.9 Hz), 119.79 (d, $^3J_{C\text{-}F}$ = 4.6 Hz).

^{19}F NMR (376 MHz, DMSO-d_6) δ –137.31.

DART-MS: *m/z* calcd for $C_{11}H_9FN_4S$ $(M+H)^+$ 249.06047, found 249.05042.

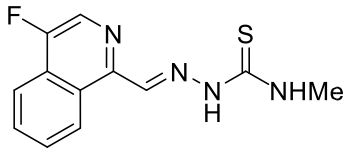
(***E***)**-2-((4-Fluoroisoquinolin-1-yl)methylene)-*N*-methylhydrazine-1-carbothioamide (2-73).** To a solution of the aldehyde **2-66b** (6.0 mg, 0.0343 mmol) in ethanol (0.5 mL) was added 4-methyl-3-thiosemicarbazide (3.6 mg, 0.0343 mmol) and HCl (34 µL, 0.206 mmol, 6 M in H_2O). The mixture was stirred and refluxed for 1.5 h then cooled to 22 °C. The hydrochloride salt that formed was then neutralized with saturated aqueous $NaHCO_3$ solution (0.5 mL). The precipitate of the desired compound was collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline **2-73** as a pale-yellow solid (2.6 mg, 29% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

11.76 (s, 1H)

9.19 (d, J = 8.6 Hz, 1H)

8.56 (d, J = 1.4 Hz, 1H)



8.56 (s, 1H) 2-73

8.34 (d, J = 4.4 Hz, 1H)

8.14 (d, J = 8.3 Hz, 1H)

7.94–7.97 (m, 1H)

7.85–7.89 (m, 1H)

3.06 (d, J = 4.6 Hz, 3H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 178.84, 154.73 (d, $^1J_{C\text{-}F}$ = 262.2 Hz), 148.03 (d, $^3J_{C\text{-}F}$ = 5.2 Hz), 145.84, 131.75, 130.69, 128.10 (d, $^2J_{C\text{-}F}$ = 23.3 Hz), 127.75, 127.35 (d, $^4J_{C\text{-}F}$ = 3.0 Hz), 126.51 (d, $^2J_{C}$F = 14.9 Hz), 119.79 (d, $^3J_{C\text{-}F}$ = 4.6 Hz), 31.86.

^{19}F NMR (376 MHz, DMSO-d_6) δ –137.53, extraneous peak found at –134.32.

DART-MS: *m/z* calcd for $C_{12}H_{12}FN_4S$ $(M+H)^+$ 263.07612, found 263.07520.

(*E*)-2-((4-Fluoroisoquinolin-1-yl)methylene)-*N*,*N*-dimethylhydrazine-1-carbothioamide and (*Z*)-2((4-Fluoroisoquinolin-1-yl)methylene)-*N*,*N*-dimethylhydrazine-1-carbothioamide (2-78). To a solution of the aldehyde **2-66b** (17.8 mg, 0.102 mmol) in methanol (1.0 mL) was added 4,4-dimethyl-3-thiosemicarbazide (12.0 mg, 0.102 mmol) and HCl (101 µL, 0.610 mmol, 6 M in H_2O). The mixture was microwaved at 300 W and 50 °C for 1 h. The hydrochloride salt that formed was then neutralized with saturated aqueous $NaHCO_3$ solution (1.0 mL). The precipitate of the desired compound was collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline **2-78** as a pale-yellow solid (16.0 mg, 57% yield) (mixture of *E* and *Z* isomers).

^{1}H NMR (500 MHz, DMSO-d_6) δ

15.52 (s, 0.15H)

11.28 (s, 1H)

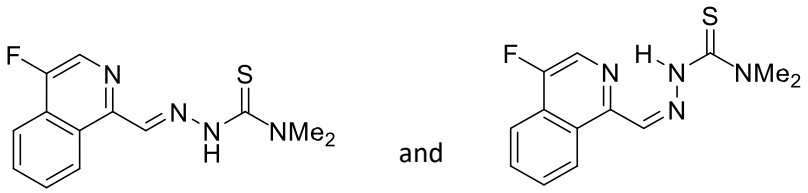
9.87 (d, *J* = 8.7 Hz, 1H)

8.87 (d, *J* = 9.0 Hz, 0.15H)

8.77 (d, *J* = 1.9 Hz, 0.15H)

8.69 (s, 1H)

8.57 (d, *J* = 1.6 Hz, 1.15H)



2-78

8.23 (d, J = 8.2 Hz, 0.15H),

8.15 (d, J = 8.2 Hz, 1H)

8.02–8.05 (m, 0.15H)

7.92–7.97 (m, 1.15H)

7.86 (ddd, J = 8.3, 6.9, 1.3 Hz, 1H)

3.42 (s, 0.90H)

3.36 (s, 6H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 180.79, 180.73, 154.60 (d, $^{1}J_{C\text{-}F}$ = 261.7 Hz), 154.13 (d, $^{1}J_{C\text{-}F}$ = 261.8 Hz), 148.75 (d, $^{3}J_{C\text{-}F}$ = 5.1 Hz), 147.76 (d, $^{3}J_{C}F$ = 5.7 Hz), 147.05, 132.52, 131.59 (d, $^{4}J_{C\text{-}F}$ = 5.1 Hz), 131.10, 130.49, 130.30, 128.60 (d, $^{4}J_{C\text{-}F}$ = 3.3 Hz), 128.41 (d, $^{4}J_{C\text{-}F}$ = 1.0 Hz), 127.90 (d, $^{2}J_{C\text{-}F}$ = 23.3 Hz), 127.19 (d, $^{4}J_{C\text{-}F}$ = 2.6 Hz), 126.88 (d, $^{2}J_{C\text{-}F}$ = 14.8 Hz), 126.71 (d, $^{2}J_{C\text{-}F}$ = 14.7 Hz), 126.35 (d, $^{2}J_{C\text{-}F}$ = 25.2 Hz),124.98, 120.23 (d, $^{3}J_{C\text{-}F}$ = 4.3 Hz), 119.79 (d, $^{3}J_{C\text{-}F}$ = 4.7 Hz), 42.04.

^{19}F NMR (376 MHz, DMSO-d_6) δ –134.93, –138.02.

DART-MS: *m/z* calcd for $C_{13}H_{14}FN_4S$ $(M+H)^+$ 277.09177, found 277.09096.

6-Fluoro-1-methylisoquinoline (65c). To a solution of 6-fluoroisoquinoline (1.00 g, 6.80 mmol) in THF (120 mL) was added allyl chloroformate (1.64 mL, 13.59 mmol). MeMgBr (6.98 mL, 13.59 mmol, 2 M in diethyl ether) was then added dropwise to the reaction mixture at 0 °C while stirring and the mixture was gradually warmed to 22 °C over a period of 2 h. The reaction was quenched with saturated aqueous NH_4Cl (12 mL) and water (120 mL) was added. The organic

layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 120 mL). The organic layers were combined and dried over $MgSO_4$, filtered, and then concentrated *in vacuo*. The crude residue in ethyl acetate was filtered through a silica plug, concentrated *in vacuo* and the crude residue was subjected to the next reaction without further purification. To a solution of the crude residue and $Pd(PPh_3)_4$ (293.4 mg, 0.254 mmol) in dichloromethane (50 mL) at 0 °C was added morpholine (437.9 uL, 5.08 mmol). The reaction was stirred and slowly warmed to 22 °C over a period of 3 h. The mixture was cooled to 0 °C and DDQ (1.15 g, 5.08 mmol) was added portionwise. After the reaction mixture stirred at 0 °C for 30 min, the reaction was slowly poured into a solution of saturated $NaHCO_3$ solution (50 mL) and extracted with dichloromethane (3 × 50). The combined extracts are washed with brine, dried over Na_2SO_4, and concentrated *in vacuo* and the crude residue was purified by flash column chromatography on silica gel (gradient, 5–25% ethyl acetate/hexanes). The isoquinoline **2-65c** was obtained as a brown oil (583.9 mg, 53% over three steps).

^{1}H NMR (500 MHz, DMSO-d_6) δ

8.37 (d, *J* = 5.8 Hz, 1H)

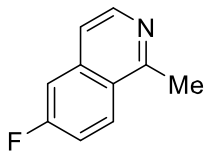
8.13 (dd, *J* = 9.2, 5.5 Hz, 1H)

7.46 (d, *J* = 5.8 Hz, 1H)

7.40 (dd, *J* = 9.3, 2.6 Hz, 1H)

7.34 (td, *J* = 8.8, 2.6 Hz, 1H)

2.95 (s, 3H).



2-65c

^{13}C NMR (125 MHz, DMSO-d_6) δ 162.94 (d, $^{1}J_{C\text{-}F}$ = 252.2 Hz), 158.53 (d, $^{5}J_{C\text{-}F}$ = 1.0 Hz), 142.77, 137.58 (d, $^{3}J_{C\text{-}F}$ = 10.4 Hz), 128.79 (d, $^{3}J_{C\text{-}F}$ = 9.6 Hz), 124.72 (d, $^{4}J_{C\text{-}F}$ = 1.0 Hz), 119.01 (d, $^{4}J_{C\text{-}F}$ = 5.0 Hz), 117.31 (d, $^{2}J_{C\text{-}F}$ = 25.0 Hz), 110.44 (d, $^{2}J_{C\text{-}F}$ = 20.6 Hz), 22.53.

^{19}F NMR (376 MHz, $CDCl_3$) δ –108.23.

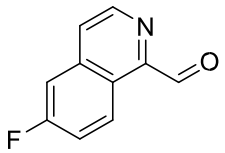
DART-MS: *m/z* calcd for $C_{10}H_9FN$ $(M+H)^+$ 162.07135, found 162.07096.

6-Fluoroisoquinoline-1-carboxaldehyde (2-66c). To a solution of the isoquinoline **2-65c** (500.0 mg, 3.10 mmol) in 1,4-dioxane (19.0 mL) was added SeO_2 (688.4 mg, 6.20 mmol). The mixture was stirred at 60 °C overnight then cooled to 22 °C. The mixture was concentrated *in vacuo* and the crude residue was purified by flash column chromatography on silica gel (gradient, 5–25% ethyl acetate/hexanes). The isoquinoline **2-66c** was obtained as a white solid (200.9 mg, 37% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

10.35 (s, 1H)

9.39 (ddd, *J* = 10.1, 5.6, 0.9 Hz, 1H)



8.75 (dd, *J* = 5.6, 0.4 Hz, 1H) 2-66c

7.85 (d, *J* = 5.5 Hz, 1H)

7.50–7.54 (m, 2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 195.51, 163.18 (d, $^{1}J_{C\text{-}F}$ = 255.1 Hz), 149.71 (d, $^{4}J_{C\text{-}F}$ = 1.8 Hz), 143.34 (d, $^{5}J_{C\text{-}F}$ = 1.0 Hz), 138.70 (d, $^{3}J_{C\text{-}F}$ = 10.4 Hz), 129.24 (d, $^{3}J_{C\text{-}F}$ = 9.2 Hz), 124.90 (d,

$^{4}J_{C\text{-}F}$ = 5.4 Hz), 123.49 (d, $^{5}J_{C\text{-}F}$ = 1.0 Hz), 120.53 (d, $^{2}J_{C\text{-}F}$ = 24.8 Hz), 110.23 (d, $^{2}J_{C\text{-}F}$ = 20.9 Hz).

^{19}F NMR (376 MHz, $CDCl_3$) δ –105.46.

DART-MS: *m/z* calcd for $C_{10}H_7FNO$ $(M+H)^+$ 176.05062, found 176.05015.

(*E*)-2-((6-Fluoroisoquinolin-1-yl)methylene)hydrazine-1-carbothioamide (2-70). To a solution of the aldehyde **2-66c** (10.2 mg, 0.0582 mmol) in ethanol (0.5 mL) was added thiosemicarbazide (5.3 mg, 0.0582 mmol) and HCl (58 µL, 0.349 mmol, 6 M in H_2O). The mixture was stirred and refluxed for 1.5 h then cooled to 22 °C. The hydrochloride salt that formed was then neutralized with saturated aqueous $NaHCO_3$ solution (0.5 mL). The precipitate of the desired compound was collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline **2-70** as a pale-yellow solid (13.4 mg, 93% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

11.74 (s, 1H)

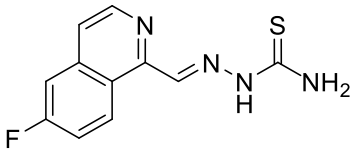
9.30 (dd, *J* = 9.4, 5.8 Hz, 1H)

8.55 (d, *J* = 5.6 Hz, 1H)

8.51 (s, 2H)

7.80–7.85 (m, 3H)

7.57 (td, *J* = 9.0, 2.8 Hz, 1H).



2-70

^{13}C NMR (125 MHz, DMSO-d_6) δ 178.88, 162.70 (d, $^{1}J_{C\text{-}F}$ = 250.4 Hz), 151.35, 146.33, 143.50, 138.62 (d, $^{3}J_{C\text{-}F}$ = 10.7 Hz), 131.52 (d, $^{3}J_{C\text{-}F}$ = 9.5 Hz), 123.24, 121.79 (d, $^{4}J_{C}$F = 5.0 Hz), 119.36 (d, $^{1}J_{C\text{-}F}$ = 24.5 Hz), 110.86 (d, $^{2}J_{C\text{-}F}$ = 20.7 Hz).

^{19}F NMR (376 MHz, DMSO-d_6) δ – 107.79, extraneous peak found at –106.49.

DART-MS: *m/z* calcd for $C_{11}H_{10}FN_4S$ $(M+H)^+$ 249.06047, found 249.05984.

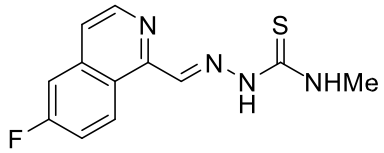
(*E*)-2-((6-Fluoroisoquinolin-1-yl)methylene)-*N*-methylhydrazine-1-carbothioamide (2-74). To a solution of the aldehyde **2-66c** (8.8 mg, 0.0502 mmol) in ethanol (0.5 mL) was added 4-methyl-3-thiosemicarbazide (5.3 mg, 0.0502 mmol) and HCl (50 µL, 0.300 mmol, 6 M in H_2O). The mixture was stirred and refluxed for 1.5 h then cooled to 22 °C. The hydrochloride salt that formed was then neutralized with saturated aqueous $NaHCO_3$ solution (0.5 mL). The precipitate of the desired compound was collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline **2-74** as a pale-yellow solid (10.8 mg, 82% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

11.80 (s, 1H)

9.20 (dd, *J* = 9.4, 5.7 Hz, 1H)

8.55 (d, *J* = 5.6 Hz, 1H)



8.54 (s, 1H)

8.35 (d, *J* = 4.7 Hz, 1H) 2-74

7.83 (dd, J = 9.2, 3.9 Hz, 2H)

7.60 (td, J = 9.0, 2.7 Hz, 1H)

3.06 (d, J = 4.5 Hz, 3H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 178.56, 162.71 (d, $^{1}J_{C\text{-}F}$ = 250.4 Hz), 151.55, 145.22, 143.53, 138.62 (d, $^{3}J_{C\text{-}F}$ = 10.6 Hz), 131.28 (d, $^{3}J_{C\text{-}F}$ = 9.5 Hz), 123.29, 121.67 (d, $^{4}J_{C\text{-}F}$ = 5.1 Hz), 119.23 (d, $^{2}J_{C\text{-}F}$ = 24.8 Hz), 110.89 (d, $^{2}J_{C\text{-}F}$ = 20.8 Hz), 31.85.

^{19}F NMR (376 MHz, DMSO-d_6) δ –106.55, extraneous peak found at –107.74.

DART-MS: *m/z* calcd for $C_{12}H_{12}FN_4S$ $(M+H)^+$ 263.07612, found 263.07538.

(*E*)-2-((6-Fluoroisoquinolin-1-yl)methylene)-*N*,*N*-dimethylhydrazine-1-carbothioamide and (*Z*)-2((6-Fluoroisoquinolin-1-yl)methylene)-*N*,*N*-dimethylhydrazine-1-carbothioamide (2-79). To a solution of the aldehyde **2-66c** (8.6 mg, 0.0491 mmol) in ethanol (0.5 mL) was added 4,4-dimethyl-3-thiosemicarbazide (5.9 mg, 0.0491 mmol) and HCl (49 µL, 0.294 mmol, 6 M in H_2O). The mixture was stirred and refluxed for 1.5 h then cooled to 22 °C. The hydrochloride salt that formed was then neutralized with saturated aqueous $NaHCO_3$ solution (0.5 mL). The precipitate of the desired compound was collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline **2-79** as a pale-yellow solid (7.4 mg, 55% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

15.90 (s, 0.21H)

11.30 (s, 1H)

9.87 (dd, *J* = 9.5, 5.9 Hz, 1H)

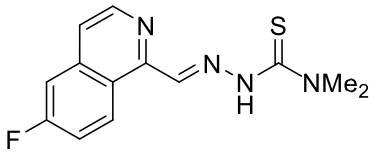
8.91 (dd, *J* = 9.4, 5.4 Hz, 0.21H)

8.66 (m, 1.21H)

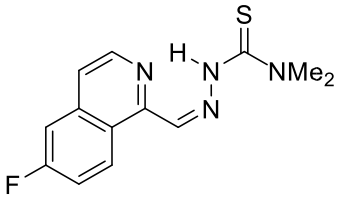
8.59 (s, 0.21H)

8.55 (d, *J* = 5.6 Hz, 1H)

7.97 (d, *J* = 5.6 Hz, 0.21H)



and



2-79

7.91 (dd, *J* = 9.6, 2.7 Hz, 0.21H)

7.79–7.82 (m, 2H)

7.73 (td, *J* = 9.1, 2.7 Hz, 0.21H)

7.62 (ddd, *J* = 9.6, 8.6, 2.8 Hz, 1H)

3.40 (s, 1.26H)

3.33 (s, 6H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 180.78, 163.19 (d, $^{1}J_{C\text{-}F}$ = 251.7 Hz), 162.66 (d, $^{1}J_{C\text{-}F}$ = 250.6 Hz), 151.99 (d, $^{5}J_{C\text{-}F}$ = 1.2 Hz), 150.63 (d, $^{5}J_{C\text{-}F}$ = 0.9 Hz), 147.57, 143.42, 141.42, 138.83 (d, $^{3}J_{C\text{-}F}$ = 15.5 Hz), 138.76 (d, $^{4}J_{C\text{-}F}$ = 10.7 Hz), 132.08 (d, $^{3}J_{C\text{-}F}$ = 9.3 Hz), 131.62, 128.64 (d, $^{3}J_{C\text{-}F}$ = 9.9 Hz), 124.14, 123.10, 122.12 (d, $^{4}J_{C\text{-}F}$ = 5.2 Hz), 121.56 (d, $^{4}J_{C\text{-}F}$ = 5.1 Hz), 119.54 (d, $^{1}J_{C\text{-}F}$ = 25.6 Hz), 119.14 (d, $^{2}J_{C\text{-}F}$ = 24.4 Hz), 111.48 (d, $^{2}J_{C\text{-}F}$ = 20.8 Hz), 110.90 (d, $^{2}J_{C\text{-}F}$ = 20.7 Hz), 42.04.

^{19}F NMR (376 MHz, DMSO-d_6) δ –106.34, –107.95.

DART-MS: *m/z* calcd for $C_{13}H_{14}FN_4S$ $(M+H)^+$ 277.09177, found 277.09098.

4-Fluoro-1-methyl-5-nitroisoquinoline (2-65b.i). To a solution of the isoquinoline **2-65b** (0.376 g, 2.333 mmol) in sulfuric acid (0.4 mL) at 0 ˚C was added KNO_3 (0.234 g, 2.333 mmol) in sulfuric acid (0.6 mL). The mixture was heated at 60 ˚C for 2 h and then poured slowly over crushed ice. The solution was made alkaline with NH_4OH; the resulting tan precipitate was filtered, washed with water, and dried to afford the isoquinoline **2-65b.i** as a tan solid (0.210 g, 44% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

8.42 (d, *J* = 2.9 Hz, 1H)

8.36 (d, *J* = 8.3 Hz, 1H)

7.98 (d, *J* = 7.4 Hz, 1H)

7.77 (t, *J* = 7.8 Hz, 1H)

3.03 (s, 3H).

2-65b.i

^{13}C NMR (125 MHz, $CDCl_3$) δ 155.10 (d, $^4J_{C\text{-}F}$ = 5.2 Hz), 151.08 (d, $^1J_{C\text{-}F}$ = 262.1 Hz), 144.92, 130.04 (d, $^2J_{C\text{-}F}$ = 25.2 Hz), 129.62, 128.88, 127.24, 125.53, 118.43 (d, $^3J_{C\text{-}F}$ = 12.1 Hz), 22.66.

^{19}F NMR (376 MHz, $CDCl_3$) δ -133.19.

DART-MS: *m/z* calcd for $C_{10}H_8FN_2O_2$ $(M+H)^+$ 207.05643, found 207.05705.

4-Fluoro-1-methylisoquinolin-5-amine (2-65b.ii). To a solution of the isoquinoline **2-65b.i** (0.210 g, 1.02 mmol) in methanol (50 mL) was added iron powder (0.171 g, 3.06 mmol) and HCl (1 mL, 12 M in H_2O). The mixture was refluxed for 2 h and then a solution of sodium hydroxide (2 mL, 6 M in H_2O) was added. The mixture was filtered and extracted with diethyl ether (200

mL). The organic layer was dried over Na_2SO_4, filtered, and then concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel (gradient, 10–30% ethyl acetate/hexanes). The amine **2-65b.ii** was obtained as a brown solid (0.173 g, 96% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

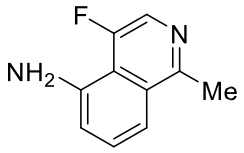
8.09 (d, *J* = 5.1 Hz, 1H)

7.46–7.39 (m, 2H)

6.88 (dd, *J* = 6.9, 1.8 Hz, 1H)

4.83 (br s, 2H)

2.87 (d, *J* = 1.3 Hz, 3H).



2-65b.ii

^{13}C NMR (125 MHz, $CDCl_3$) δ 155.92 (d, $^1J_{C\text{-}F}$ = 253.3 Hz), 154.74 (d, $^4J_{C\text{-}F}$ = 4.9 Hz), 142.23 (d, $^4J_{C\text{-}F}$ = 3.0 Hz), 129.19, 115.79 (d, $^3J_{C\text{-}F}$ = 8.8 Hz), 114.77, 114.76, 113.81, 22.61.

^{19}F NMR (376 MHz, $CDCl_3$) δ -136.45.

DART-MS: *m/z* calcd for $C_{10}H_{10}FN_2$ $(M+H)^+$ 177.08225, found 177.08220.

***tert*-Butyl (4-fluoro-1-methylisoquinolin-5-yl)carbamate (2-65b.iii).** To a solution of the amine **2-65b.ii** (1.14 g, 6.49 mmol) in THF (15 mL) was added DMAP (79.3 mg, 0.65 mmol) then Boc_2O (3.54 g, 16.23 mmol) and the mixture was stirred at 22 °C overnight. After completion of the reaction as judged by TLC, K_2CO_3 (2.69 g, 19.47 mmol) and methanol (10 mL) were added to the reaction mixture and then refluxed overnight. The mixture was then concentrated *in vacuo* and resuspended in ethyl acetate (20 mL) and water (20 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The

organic layers were combined and dried over Na_2SO_4, filtered, and then concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel (gradient, 5–20% ethyl acetate/hexanes). The carbamate **2-65b.iii** was obtained as a brown oil (0.572 g, 33% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

8.50 (dd, *J* = 7.9, 1.0 Hz, 1H)

8.17 (d, *J* = 5.7 Hz, 1H)

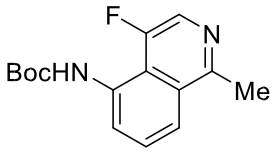
8.05 (d, *J* = 7.8 Hz, 1H)

7.73 (ddd, *J* = 8.4, 3.0, 1.0 Hz, 1H)

7.60 (t, *J* = 8.2 Hz, 1H)

2.88 (d, *J* = 1.3 Hz, 3H)

1.55 (s, 9H).



2-65b.iii

^{13}C NMR (125 MHz, $CDCl_3$) δ 158.37 (d, $^1J_{C\text{-}F}$ = 296.5 Hz), 153.19, 137.68, 131.10, 128.23 (d, $^3J_{C\text{-}F}$ = 10.7 Hz), 124.83, 124.45, 121.14 (d, $^2J_{C\text{-}F}$ = 22.3 Hz), 119.58, 82.72, 28.15, 17.84, one low-field carbon was either not observed or is overlapping with another low-field carbon.

^{19}F NMR (376 MHz, $CDCl_3$) δ -136.85.

DART-MS: *m/z* calcd for $C_{15}H_{18}FN_2O_2$ $(M+H)^+$ 277.13468, found 277.13425.

***tert*-Butyl methyl(4-fluoro-1-methylisoquinolin-5-yl)carbamate (2-65b.iv).** To a solution of the isoquinoline **2-65b.iii** (0.524 g, 1.90 mmol) in THF (10 mL) was added sodium hydride, 60% in mineral oil (59.2 mg, 2.49 mmol). After effervescence ceased, the resulting solution was

refluxed for 30 min. To the reaction mixture was then added methyl iodide (0.350 g, 4.49 mmol) in THF (2 mL) and the solution refluxed overnight. The mixture was concentrated and passed through a silica plug (gradient, 10–66% ethyl acetate/hexanes). The mixture was concentrated *in vacuo* and the crude residue was purified by flash column chromatography (gradient, 10–30% EtOAc:hexanes). The isoquinoline **2-65b.iv** was obtained as an amber oil containing a mixture of rotamers (0.456 g, 82% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ

8.25–8.22 (m, 1.5H)

8.11–8.02 (m, 1.5H)

7.71–7.61 (m, 2H)

7.55 (dd, *J* = 7.3, 1.3 Hz, 1H)

3.28 (s, 3H)

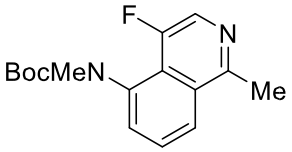
3.27 (s, 1.5H)

2.96 (s, 3H)

2.95 (s, 1.5H)

1.53 (s, 4.5H)

1.21 (s, 9H).



2-65b.iv

^{13}C NMR (125 MHz, $CDCl_3$) δ 155.41, 154.92 (d, $^{4}J_{C\text{-}F}$ = 5.7 Hz), 154.90 (d, $^{4}J_{C\text{-}F}$ = 5.4 Hz), 154.63, 154.51, 153.54 (d, $^{1}J_{C\text{-}F}$ = 259.3 Hz), 137.94, 131.60, 130.52, 130.05, 129.74, 128.44, 128.19, 127.82 (d, $^{2}J_{C\text{-}F}$ = 27.6 Hz), 125.49, 125.08, 124.53, 124.34 (d, $^{3}J_{C\text{-}F}$ = 8.1 Hz), 80.79,

80.23, 38.52 (d, $^{5}J_{C\text{-}F}$ = 3.82 Hz), 37.81 (d, $^{5}J_{C\text{-}F}$ = 3.07 Hz), 28.38, 28.05, 22.46, 22.26, two low-field carbons were either not observed or are overlapping with another low-field carbon.

^{19}F NMR (376 MHz, $CDCl_3$) δ -140.37, -141.22.

DART-MS: *m/z* calcd for $C_{16}H_{20}FN_2O_2$ $(M+H)^+$ 291.15033, found 291.14981.

***tert*-Butyl methyl(4-fluoro-1-formylisoquinolin-5-yl)carbamate (2-68b).** To a solution of the isoquinoline **2-65b.iv** (0.40 g, 1.38 mmol) in 1,4-dioxane (10 mL) was added SeO_2 (0.183 g, 1.65 mmol). The mixture was stirred at 60 °C overnight then cooled to 22 °C. The mixture was concentrated *in vacuo* and the crude residue was purified by flash column chromatography on silica gel (gradient, 5–25% ethyl acetate/hexanes). The aldehyde **2-68c** was obtained as an off-white solid containing a mixture of rotamers (0.152 g, 36% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

10.32 (d, *J* = 1.5 Hz, 1H)

10.29 (d, *J* = 1.6 Hz, 0.5H)

9.38 (tdd, *J* = 7.5, 2.7, 1.4 Hz, 1.5H)

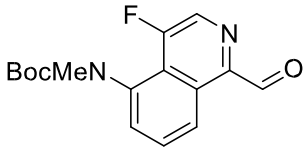
8.58 (dd, *J* = 3.9, 1.1 Hz, 1H)

8.56 (dd, *J* = 4.0, 1.3 Hz, 0.5H)

7.80 (t, *J* = 7.3, 1.4 Hz, 1.5H)

7.68 (dt, *J* = 7.5, 0.5Hz, 0.5H)

7.62 (dt, *J* = 7.4, 1.0 Hz, 1H)



2-68c

3.31 (d, J = 1.1 Hz, 3H)

3.30 (d, J = 0.9 Hz, 1.5H)

1.54 (s, 4.5H)

1.21 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$) 194.12, 194.09, 156.39 (d, $^1J_{C\text{-}F}$ = 272.5 Hz), 156.35 (d, $^1J_{C\text{-}F}$ = 255.9 Hz), 155.21, 154.41, 146.67 (d, $^3J_{C\text{-}F}$ = 6.1 Hz), 137.64 (d, $^4J_{C\text{-}F}$ = 1.7 Hz), 131.73 (d, $^4J_{C\text{-}F}$ = 1.9 Hz), 131.12 (d, $^4J_{C\text{-}F}$ = 1.9 Hz), 131.05, 130.95 (d, $^2J_{C\text{-}F}$ = 21.5 Hz), 130.85, 130.57 (d, $^2J_{C\text{-}F}$ = 28.5 Hz), 130.25 (d, $^2J_{C\text{-}F}$ = 28.2 Hz), 129.83 (d, $^4J_{C\text{-}F}$ = 2.4 Hz), 129.49 (d, $^4J_{C\text{-}F}$ = 2.5 Hz), 125.05, 124.68 (d, $^4J_{C\text{-}F}$ = 1.7 Hz), 124.56 ($^3J_{C\text{-}F}$, J = 7.4 Hz), 124.44 (d, $^3J_{C\text{-}F}$ = 7.1 Hz), 81.01, 80.49, 38.56 (d, $^5J_{C\text{-}F}$ = 3.3 Hz), 37.83 (d, $^5J_{C\text{-}F}$ = 2.6 Hz), 28.36, 28.03, one low-field carbon was either not observed or is overlapping with another low-field carbon.

^{19}F NMR (376 MHz, $CDCl_3$) δ -133.9.

DART-MS: *m/z* calcd for $C_{16}H_{18}FN_2O_3$ $(M+H)^+$ 305.12960, found 305.12824.

(*E*)-2-((4-Fluoro-5-(methylamino)isoquinolin-1-yl)methylene)-*N*,*N*-dimethylhydrazine-1carbothioamide and (*Z*)-2-((4-Fluoro-5-(methylamino)isoquinolin-1-yl)methylene)-*N*,*N*dimethylhydrazine-1-carbothioamide (2-81). To a solution of the aldehyde **2-68b** (30.0 mg, 0.099 mmol) in methanol (3.0 mL) was added 4,4-dimethyl-3-thiosemicarbazide (11.7 mg, 0.985 mmol) and HCl (98 µL, 0.59 mmol, 6 M in H_2O). The mixture was microwaved at 300 W and 50 °C for 1 h. The hydrochloride salt that formed was then neutralized with saturated aqueous $NaHCO_3$ solution (1.5 mL). The precipitate of the desired compound was collected by filtration,

washed with water and ethanol and then dried to yield the isoquinoline **2-81** as a pale-yellow solid containing a mixture of *E*- and *Z*-isomers (12.2 mg, 41% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

15.46 (s, 0.33H)

11.13 (br s, 1H)

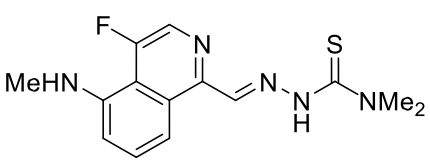
8.91 (dd, *J* = 8.4, 2.9 Hz, 1H)

8.62 (s, 1H)

8.50 (d, *J* = 5.1 Hz, 0.33H)

8.39 (s, 0.33H)

8.32 (d, *J* = 4.8 Hz, 1H)



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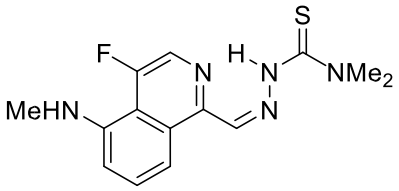


7.89 (dd, *J* = 8.5, 2.9 Hz, 0.33H)

7.65 (t, *J* = 8.2 Hz, 0.33H)

2-81

7.57 (t, *J* = 8.2 Hz, 1H)

6.82 (d, *J* = 8.0 Hz, 0.33H)

6.73 (d, *J* = 7.9 Hz, 1H)

6.55 (dd, *J* = 11.9, 5.2 Hz, 0.33H)

6.39 (dd, *J* = 12.4, 5.0 Hz, 1H)

3.37 (s, 1.98H)

3.31 (s, 6H)

2.86–2.84 (m, 3.99H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 180.95, 180.72, 156.41 (d, J = 260.4 Hz), 147.99 (d, $^4J_{C\text{-}F}$ = 4.3 Hz), 147.41, 147.16, 144.92, 144.61 (d, $^4J_{C\text{-}F}$ = 3.7 Hz), 131.90, 131.69, 130.83, 130.78, 129.29 (d, $^4J_{C}F$ = 2.4 Hz), 127.41 (d, $^2J_{C\text{-}F}$ = 28.8 Hz), 125.43 (d, $^2J_{C\text{-}F}$ = 30.5 Hz), 115.98 (d, $^2J_{C\text{-}F}$ = 7.6 Hz), 113.89, 113.84, 110.26, 108.50, 107.78, 42.15, 30.95, one low-field carbon was either not observed or is overlapping with another low-field carbon.

^{19}F NMR (376 MHz, DMSO-d_6) δ -125.86, -129.02.

DARTMS: *m/z* calcd for $C_{14}H_{17}FN_5S$ $(M+H)^+$ 306.11832, found 306.11716.

6-Fluoro-1-methyl-5-nitroisoquinoline (2-65c.i). To a solution of the isoquinoline **2-65c** (0.584 g, 3.623 mmol) in sulfuric acid (0.8 mL) at 0 ˚C was added KNO_3 (0.366 g, 3.623 mmol) in sulfuric acid (1.2 mL). The mixture was heated at 60 ˚C for 2 h and then poured slowly over crushed ice. The solution was made alkaline with NH_4OH; the resulting tan precipitate was filtered, washed with water, and dried to afford the isoquinoline **2-65c.i** as a tan solid (0.264 g, 35% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

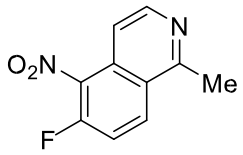
8.58 (d, J = 6.1 Hz, 1H)

8.41 (dd, J = 9.4, 4.9 Hz, 1H)

7.70 (d, J = 6.0 Hz, 1H)

7.55 (t, J = 9.2 Hz, 1H)

3.07 (s, 3H).



2-65c.i

^{13}C NMR (125 MHz, $CDCl_3$) δ 159.08, 155.06 (d, $^1J_{C\text{-}F}$ = 266.6 Hz), 144.50, 132.29 (d, $^3J_{C\text{-}F}$ = 10.0 Hz), 129.83, 124.19, 117.40 (d, $^2J_{C\text{-}F}$ = 23.5 Hz), 113.60, 22.41.

^{19}F NMR (376 MHz, $CDCl_3$) δ -113.01.

DART-MS: *m/z* calcd for $C_{10}H_8FN_2O_2$ $(M+H)^+$ 207.05643, found 207.05690.

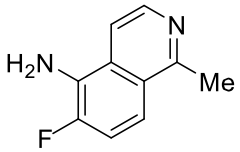
6-Fluoro-1-methylisoquinolin-5-amine (2-65c.ii). To a solution of the isoquinoline **2-65c.i** (0.264 g, 1.28 mmol) in methanol (60 mL) was added iron powder (0.214 g, 3.83 mmol) and HCl (1 mL, 12 M in H_2O). The mixture was refluxed for 2 h and then a solution of sodium hydroxide (2 mL, 6 M in H_2O) was added. The mixture was filtered and extracted with diethyl ether (200 mL). The organic layer was dried over Na_2SO_4, filtered, and then concentrated *in vacuo*. The crude residue was purified by flash column chromatography (gradient, 10–30% ethyl acetate/hexanes). The amine **2-65c.ii** was obtained as a brown solid (145.8 mg, 82% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

8.36 (d, *J* = 6.2 Hz, 1H)

7.62 (m, 2H)

7.44 (d, *J* = 9.9 Hz, 1H)



2-65c.ii

4.27 (br s, 2H)

3.06 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 164.80, 159.77 (d, $^1J_{C\text{-}F}$ = 263.7 Hz), 150.34, 139.41 (d, $^3J_{C\text{-}F}$ = 10.8 Hz), 137.94, 133.78, 128.91, 122.74 (d, $^2J_{C\text{-}F}$ = 22.7 Hz), 118.04 (d, $^4J_{C\text{-}F}$ = 5.2 Hz), 27.69.

^{19}F NMR (376 MHz, $CDCl_3$) δ -125.82.

DART-MS: *m/z* calcd for $C_{10}H_{10}FN_2$ $(M+H)^+$ 177.08225, found 177.08291.

***tert*-Butyl (6-fluoro-1-methylisoquinolin-5-yl)carbamate (2-65c.iii).** To a solution of the amine **2-65c.ii** (0.715 g, 4.06 mmol) in THF (15 mL) was added DMAP (49.5 mg, 0.41 mmol) then Boc_2O (2.21 g, 10.14 mmol) and the mixture was stirred at 22 °C overnight. After completion of the reaction as attested by TLC, K_2CO_3 (1.68 g, 12.17 mmol) and methanol (10 mL) were added to the reaction mixture and was refluxed overnight. The mixture was then concentrated *in vacuo* and resuspended in ethyl acetate (20 mL) and water (20 mL). The organic layer was separated and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined and dried over Na_2SO_4, filtered, and then concentrated *in vacuo*. The crude residue was purified by flash column chromatography on silica gel (gradient, 5–20% ethyl acetate/hexanes). The isoquinoline **2-65c.iii** was obtained as a brown oil (0.303 g, 27% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

8.40 (d, *J* = 6.0 Hz, 1H)

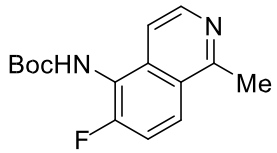
8.06 (dd, *J* = 9.3, 5.0 Hz, 1H)

7.65 (d, *J* = 6.0 Hz, 1H)

7.39 (t, *J* = 9.3 Hz, 1H)

6.59 (br s, 1H)

2.95 (s, 3H)



2-65c.iii

1.50 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 160.58 (d, $^1J_{C\text{-}F}$ = 260.2 Hz), 157.19, 153.19, 137.68, 131.09 (d, $^4J_{C\text{-}F}$ = 4.9 Hz), 128.23 (d, $^3J_{C\text{-}F}$ = 10.7 Hz), 124.83, 124.45, 121.14 (d, $^2J_{C\text{-}F}$ = 22.1 Hz), 119.60, 82.72, 28.15, 17.84.

^{19}F NMR (376 MHz, $CDCl_3$) δ -112.86.

DART-MS: *m/z* calcd for $C_{15}H_{18}FN_2O_2$ $(M+H)^+$ 277.13468, found 277.13425.

***tert*-Butyl methyl(6-fluoro-1-methylisoquinolin-5-yl)carbamate (2-65c.iv).** To a solution of the isoquinoline **2-65c.iii** (0.150 g, 0.543 mmol) in THF (4 mL) was added sodium hydride, 60% in mineral oil (28.0 mg, 0.706 mmol). After effervescence ceased, the resulting solution was refluxed for 30 min. To the reaction mixture was added the methyl iodide (0.10 g, 0.706 mmol) in THF (0.5 mL) and the solution refluxed overnight. The mixture was concentrated and passed through a silica plug (gradient, 10–66% ethyl acetate/hexanes). The mixture was concentrated *in vacuo* and the crude residue was purified by flash column chromatography (gradient, 10–30% ethyl acetate/hexanes). The isoquinoline **2-65c.iv** was obtained as a mixture of rotational isomers as an amber oil (0.120 g, 76% yield).

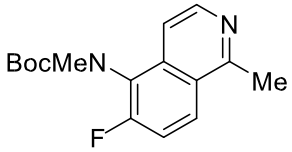
^{1}H NMR (500 MHz, $CDCl_3$) δ

8.44 (d, *J* = 5.8 Hz, 1.27H)

8.16–8.02 (m, 1.27H)

7.51 (d, *J* = 6.0 Hz, 1H)

7.49 (d, *J* = 6.3 Hz, 0.27H)



2-65c.iv

7.39 (t, J = 9.3 Hz, 1.27H)

3.26 (s, 0.81H)

3.25 (s, 3H)

2.98 (s, 3H)

2.96 (s, 0.81H)

1.56 (s, 2.43H)

1.26 (s, 9H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 158.89, 158.88, 158.09 (d, $^1J_{C\text{-}F}$ = 254.3 Hz), 154.97, 154.78, 143.18, 135.55, 135.36 (d, $^4J_{C\text{-}F}$ = 3.7 Hz), 127.66 (d, $^3J_{C\text{-}F}$ = 9.7 Hz), 127.38 (d, $^3J_{C\text{-}F}$ = 9.6 Hz), 125.25, 125.06, 124.88, 124.78, 117.42 (d, $^2J_{C\text{-}F}$ = 24.0 Hz), 117.12 (d, $^2J_{C\text{-}F}$ = 24.1 Hz), 114.35 (d, $^3J_{C\text{-}F}$ = 5.8 Hz), 81.18, 80.61, 37.43, 36.39, 28.35, 27.99, 22.62, 22.56, one low-field carbon was either not observed or is overlapping with another low-field carbon.

^{19}F NMR (376 MHz, $CDCl_3$) δ -114.54, -115.33.

DART-MS: *m/z* calcd for $C_{16}H_{20}FN_2O_2$ $(M+H)^+$ 291.15033, found 291.15011.

***tert*-Butyl methyl(6-fluoro-1-formylisoquinolin-5-yl)carbamate (2-68c).** To a solution of the isoquinoline **2-65c.iv** (0.1000 g, 0.344 mmol) in 1,4-dioxane (2 mL) was added SeO_2 (38.2 mg, 0.344 mmol). The mixture was stirred at 60 °C overnight then cooled to 22 °C. The mixture was concentrated *in vacuo* and the crude residue was purified by flash column chromatography (gradient, 5–25% EtOAc:hexanes). The aldehyde **2-68c** was obtained as an off-white solid containing a mixture of rotamers (45.3 mg, 43% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ

10.37 (s, 1H)

10.35 (s, 0.3H)

9.35 (dd, *J* = 9.4, 5.1 Hz, 1.3H)

8.82 (d, *J* = 5.8 Hz, 1.3H)

7.92 (d, *J* = 5.7 Hz, 1H)

7.88 (d, *J* = 5.8 Hz, 0.3H)

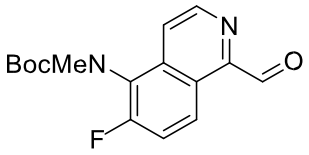
7.56 (t, *J* = 9.4 Hz, 1.3H)

3.29 (s, 0.9H)

3.28 (s, 3H)

1.57 (s, 2.7H)

1.25 (s, 9H).



2-68c

^{13}C NMR (125 MHz, $CDCl_3$) δ 195.40, 158.41 (d, $^{1}J_{C\text{-}F}$ = 257.6 Hz), 154.77, 149.92, 143.81, 143.72, 136.73 (d, $^{4}J_{C\text{-}F}$ = 4.6 Hz), 136.59 (d, $^{4}J_{C\text{-}F}$ = 3.8 Hz), 128.17 (d, $^{3}J_{C\text{-}F}$ = 10.7 Hz), 127.91 (d, $^{3}J_{C\text{-}F}$ = 9.3 Hz), 124.72 (d, $^{3}J_{C\text{-}F}$ = 13.3 Hz), 124.00, 123.80, 120.68 (d, $^{2}J_{C\text{-}F}$ = 24.7 Hz), 120.44 (d, $^{2}J_{C\text{-}F}$ = 24.1 Hz), 120.31, 120.22 (d, $^{3}J_{C\text{-}F}$ = 6.3 Hz), 81.55, 81.00, 37.58, 36.53, 28.32, 27.97, two low-field carbon were either not observed or are overlapping with another low-field carbon.

^{19}F NMR (376 MHz, $CDCl_3$) δ -112.18, -112.95.

DART-MS: *m/z* calcd for $C_{16}H_{18}FN_2O_3$ $(M+H)^+$ 305.1296, found 305.12819.

(*E*)-2-((6-Fluoro-5-(methylamino)isoquinolin-1-yl)methylene)-*N*,*N*-dimethylhydrazine-1carbothioamide and (*Z*)-2-((6-Fluoro-5-(methylamino)isoquinolin-1-yl)methylene)-*N*,*N*dimethylhydrazine-1-carbothioamide (2-80). To a solution of the aldehyde **2-68b** (10.0 mg, 0.033 mmol) in ethanol (0.5 mL) was added 4,4-dimethyl-3-thiosemicarbazide (3.9 mg, 0.033 mmol) and HCl (33 µL, 0.197 mmol, 6 M in H_2O). The mixture was stirred and refluxed for 1.5 h then cooled to 22 °C. The hydrochloride salt that formed was then neutralized with saturated aqueous $NaHCO_3$ solution (0.5 mL). The precipitate of the desired compound was collected by filtration, washed with water and ethanol and then dried to yield the isoquinoline **2-80** as a pale-yellow solid (6.7 mg, 67% yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ

15.96 (s, 0.17H)

11.22 (br s, 1H)

9.20 (s, 1H)

8.62–8.54 (m, 1.17H)

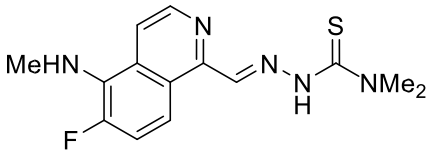
8.52 (s, 0.17H)

8.34 (d, J = 5.5 Hz, 1H)

8.20 (d, J = 6.2 Hz, 0.17H)

8.07 (dd, J = 9.3, 4.2 Hz, 0.17H)

7.87 (br s, 1H)



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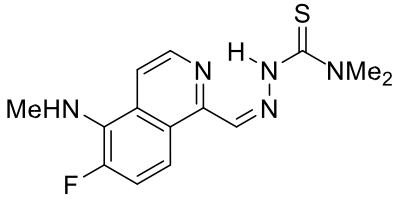

2-80

7.56 (dd, *J* = 13.6, 9.2 Hz, 0.17H)

7.33 (dd, *J* = 13.4, 9.5 Hz, 1H)

6.10 (br s, 0.17H)

5.69 (br s, 1H)

3.41 (s, 1.02H)

3.27 (s, 6H)

3.10 (t, *J* = 5.5 Hz, 0.51H)

3.05 (t, *J* = 5.2 Hz, 3H).

^{19}F NMR (376 MHz, $CDCl_3$) δ 129.05, -129.53.

DART-MS: *m/z* calcd for $C_{14}H_{17}FN_5S$ $(M+H)^+$ 305.11832, found 305.11719.

Cell culture and culture conditions

Pancreatic adenocarcinoma cell lines: PATU8988T, MIAPACA2, SU8686, PSN1, HPAC, BXPC3, DANG, SUIT2, A13A, CAPAN2, T3M4, A2.1, HUPT4, XWR200, L36PL, YAPC, PANC0327, PANC1, PATU8902, HPAF11, ASPC1, PANC0813, PANC0203, HS766T, SW1990, and CFPAC1; prostate cancer cell lines: 22Rv1, LNCaP, RM1 and C4-2; and small cell lung carcinoma cell lines: NCI-H526, NCI-H146, and NCI-H1963 were obtained from American Type Culture Collection (ATCC). 143 BTK WT and 143 BTK ρ_0, BJ WT and BJ ρ_0 cells were gifts from Prof. Michael Teitell in UCLA. Murine Prostate cancer cell lines MyC CaP was a kind gift from Prof. DLJ Thorek at WUSTL. Murine Pancreatic cancer cells KP4662 was kind gift from Prof.

Robert Vonderheide at UPenn. With a few exceptions, cell lines were cultured in DMEM (Corning) or RPMI (Corning) containing 10% fetal bovine serum (FBS, Omega Scientific) and were grown at 37 °C, 20% O_2 and 5% CO_2. All cultured cells were incubated in antibiotic free media and were regularly tested for mycoplasma contamination using MycoAlert kit (Lonza) following the manufacturer's instructions, except that the reagents were diluted 1:4 from their recommended amount.

Proliferation assay

Cells were plated in 384-well plates (500 cells/well for adherent cell lines in 30 µl volume). Drugs were serially diluted to the desired concentrations and an equivalent volume of DMSO was added to vehicle control. Following 72 h incubation, ATP content was measured using CellTiter-Glo reagent according to manufacturer's instructions (Promega, CellTiter-Glo Luminescent Cell Viability Assay), and analyzed by SpectraMax luminometer (Molecular Devices). IC_{50} and IC_{90} values, concentrations required to inhibit proliferation by 50% and 90% respectively compared to DMSO treated cells, were calculated using Prism 6.0 h (Graphpad Software).

Western blot

Cells were lysed using RIPA buffer supplemented with protease (ThermoFisher, 78,430) and phosphatase (ThermoFisher, 78,420) inhibitors, scraped, sonicated, and centrifuged (20,000 × *g* at 4 °C). Protein concentrations in the supernatant were determined using the Micro BCA Protein Assay kit (Thermo), and equal amounts of protein were resolved on pre-made Bis-Tris polyacrylamide gels (Life Technologies). Primary antibodies: $pAMPK_{T172}$ (Cell signaling, #2535, 1:1000), HO-1 (Cell signaling, #5061S, 1:1000), pS345 CHEK1(Cell signaling, #2348L, 1:1000), pT68 CHEK2 (Cell signaling, #2197 S, 1:1000), pS139 H2A.X (Millipore, 05-636, 1:1000), clvd. Casp3 (Cell signaling, #9662, 1:1000), and anti-actin (Cell Signaling Technology, 9470,

1:10,000). Primary antibodies were stored in 5% BSA (Sigma-Aldrich) and 0.1% NaN_3 in TBST solution. Anti-rabbit IgG HRP-linked (Cell Signaling Technology, 7074, 1:2500) and anti-mouse IgG HRP-linked (Cell Signaling Technology, 7076, 1:2500) were used as secondary antibodies. Chemiluminescent substrates (ThermoFisher Scientific, 34,077 and 34,095) and autoradiography film (Denville) were used for detection.

Viability/Apoptosis assay

Viable cells were measured by Trypan blue staining using vi-cell counter (Beckman Coulter, CA, USA). Apoptosis and cell death were assayed using Annexin V-FITC and PI according to manufacturer's instructions (FITC Annexin V Apoptosis Detection Kit, BD Sciences, #556570).

ROS Measurements

Cellular ROS measurement was assayed with CM-H2DCFDA staining after treatment according to manufacturer's instructions (Reactive Oxygen Species (ROS) Detection Reagents, Invitrogen, #D399). The cells were then incubated with 5 μM of CM-H2DCFDA for 30 min, spun down at 450 x g for 4 mins, and the supernatant was replaced with fresh media containing lethal compounds and/or Cu(II). Then, the cells were incubated for 30 mins, spun down, and the supernatant was replaced with PBS. The samples were analyzed using flow cytometry.

Intracellular Cu(II) measurement

Cells were plated in 6-well plates and cultured for one day. Vehicle of HCT-13 were added to the cells the following day and incubated for 24 hours. The plates were then washed 2 times with PBS containing 1 mM EDTA and 2 times with PBS alone. The concentration of Cu(II) was measured using Inductive Coupled Plasma Mass Spectrometry (ICP-MS) using standard procedure.

FACS analyses

All flow cytometry data were acquired on a five-laser LSRII cytometer (BD), and analyzed using the FlowJo software (Tree Star).

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