Aminoisoquinoline benzamides, FLT3 and Src-family kinase inhibitors potently inhibit proliferation of acute myeloid leukemia cell lines

Aim: Mutated or overexpressed FLT3 drives about 30% of reported acute myeloid leukemia (AML). Currently, FLT3 inhibitors have shown durable clinical responses but a complete remission of AML with FLT3 inhibitors remains elusive due to mutation-driven resistance mechanisms. The development of FLT3 inhibitors that also target other downstream oncogenic kinases may combat the resistance mechanism. **Results:** 4-substituted aminoisoquinoline benzamides potently inhibit Src-family kinases and FLT3, including secondary mutations, such as FLT3D835. Modifications of aminoisoquinoline benzamide to aminoquinoline or aminoquinazoline abrogated FLT3 and Src-family kinase binding. **Conclusion:** The lead aminoisoquinolines potently inhibited FLT3-driven AML cell lines, MV4–11 and MOLM-14. These aminoisoquinoline benzamides represent new kinase scaffolds with high potential to be translated into anticancer agents.

Elizabeth Larocque[‡,1], N Naganna[‡,1], Xiaochu Ma[‡,1,2], Clement Opoku-Temeng[1,2], Brandon Carter-Cooper[3], Gaurav Chopra[1,4,5], Rena G Lapidus[3] & Herman O Sintim*[,1,4,5]

[1]Institute for Drug Discovery, Purdue University, West Lafayette, IN 47907, USA
[2]On the Chemistry & Biochemistry Graduate Program, University of Maryland, College Park, MD 20742, USA
[3]Translational Core Laboratory, University of Maryland Greenebaum Cancer Center, 655 W Baltimore Street, Baltimore, MD 21201, USA
[4]Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA
[5]Purdue University Center for Cancer Research, Purdue University, West Lafayette, IN 47907, USA
*Author for correspondence:
Tel.: +1 765 496 6078
hsintim@purdue.edu
[‡]Authors contributed equally

Receptor tyrosine kinases (RTKs) regulate many key biological processes in the cell. Disruption of RTK signaling results in loss of cell homeostasis resulting in cancer and death [1]. For example, RTK-driven cancers are a result of increased expression of many RTKs, activating mutations or fusions that can lead to uncontrolled cellular proliferation [2]. Over the past few years, many tyrosine kinase inhibitors (TKIs) have been identified and used successfully in the clinic to combat different forms of cancers [3]. Imatinib (Gleevec®), an RTK inhibitor (TKI) of BCR-ABL and c-Kit, is a classic example that revolutionized the treatment of chronic myeloid leukemia (CML), Philadelphia chromosome positive (Ph+) acute lymphoblastic leukemia (Ph+ ALL) and gastrointestinal stromal tumors [4]. Response rates to many TKIs are high for the first few months, but over time secondary mutations emerge that render

the cancer resistant to the TKI [5]. For example, T315I mutations in ABL1 are resistant to imatinib and many other ABL1 inhibitors [5]. Therefore, there is still a need for newer mutation-resistant generation of TKIs.

We have developed novel inhibitors for the FLT3 receptor, which is an important class III RTK and expressed on hematopoietic progenitor cells to regulate hematopoiesis [6]. The extracellular domain of the FLT3 RTK binds FLT3 ligands and upon binding, the receptor homodimerizes and autophosphorylates (activation) in the plasma membrane [7]. Phosphorylated FLT3 activates numerous cytoplasmic effector molecules, via phosphorylation. These phosphorelays lead to hematopoietic cells differentiation and proliferation [8]. Mutations or the overexpression of FLT3 RTK leads to deregulated hematopoiesis, as seen in acute myeloid leukemia (AML) and some ALL [9,10].
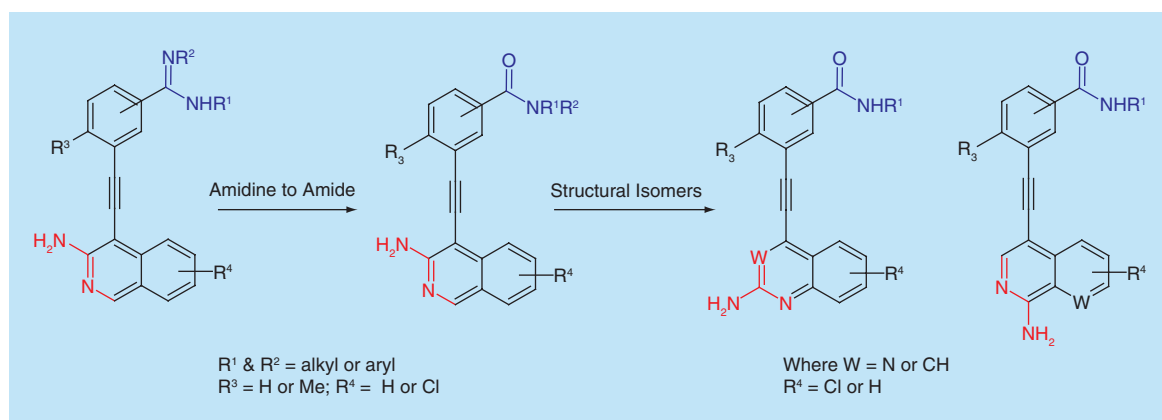
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Figure 1. The 4-substituted isoquinolines, quinolones and quinazolines synthesized for this study.

About 30% of AML patients carry the mutated form of FLT3 RTK, thus making FLT3 a prime target for anti-AML therapeutics [11]. In the past decade, there have been concerted efforts by both industry and academia to develop FLT3 inhibitors and several compounds that potently inhibit FLT3 (with nanomolar K_ds or IC_{50} values), such as midostaurin (PKC412), quizartinib, sorafenib, crenolanib and gilteritinib, have been described [12,13]. Some of these compounds have proceeded to clinical trials. Midostaurin performed well in a recently concluded Phase III clinical trial and could become one of the early FLT3 inhibitors to be approved for clinical use. Despite the successes experienced with FLT3 TKIs, none of the tested compounds have achieved a cure or long-term remission of the disease. The majority of the FLT3 inhibitors reported to date manage to clear AML in blood but do not completely eliminate AML in bone marrow [14]. Thus, over time, AML evolves and drug-resistant clones emerge in the bone marrow. Secondary FLT3 mutations, such as D835 and F691, are problematic and resistant to many FLT3 TKI developed to date [15]. In addition to secondary kinase

mutations that render targeted TKIs ineffective, the clonal evolution and heterogeneity of AML make it challenging to cure [16]. This contrasts with CML, which has a relatively simple biology and is largely driven by the BCR-ABL and hence can be potentially cured with TKIs.

Results & discussion

We recently noted that 3-aminoisoquinoline analogs that are substituted at the 4 position with benzamidine, inhibit FLT3 kinase and potently inhibit the proliferation of FLT3-driven AML cell lines, such as MV4–11 and MOLM-14 [17]. Multiple amidine drugs, such as viramidine (a potential antiviral drug [18]), are used clinically or have gone through clinical trials. However, these drugs are generally not orally bioavailable and therefore are typically administered intravenously. For oral bioavailability, amidine drugs are traditionally converted into less basic prodrugs [19]. Benzamides, on the other hand, are orally bioavailable and therefore we embarked on structure–activity relationship (SAR) studies to identify 4-benzamide substituted 3-aminoisoquinoline and structural mimics (Figure 1) as
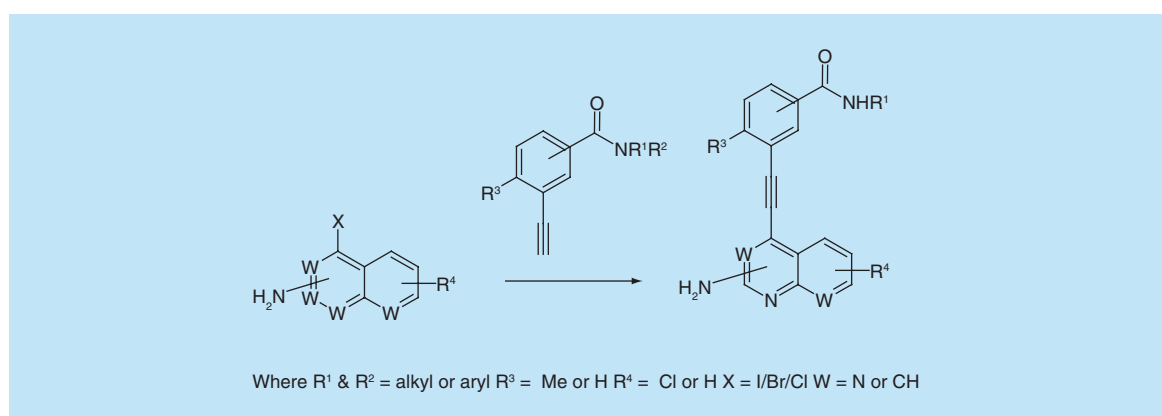

Figure 2. Synthesis of target compounds via Sonogashira coupling. Condition: Pd(PPh₃)₂Cl₂ (5 mol%), CuI (5 mol%), PPh₃ (0.1 equiv.), triethylamine (22 equiv.), 50°C, 12 h.
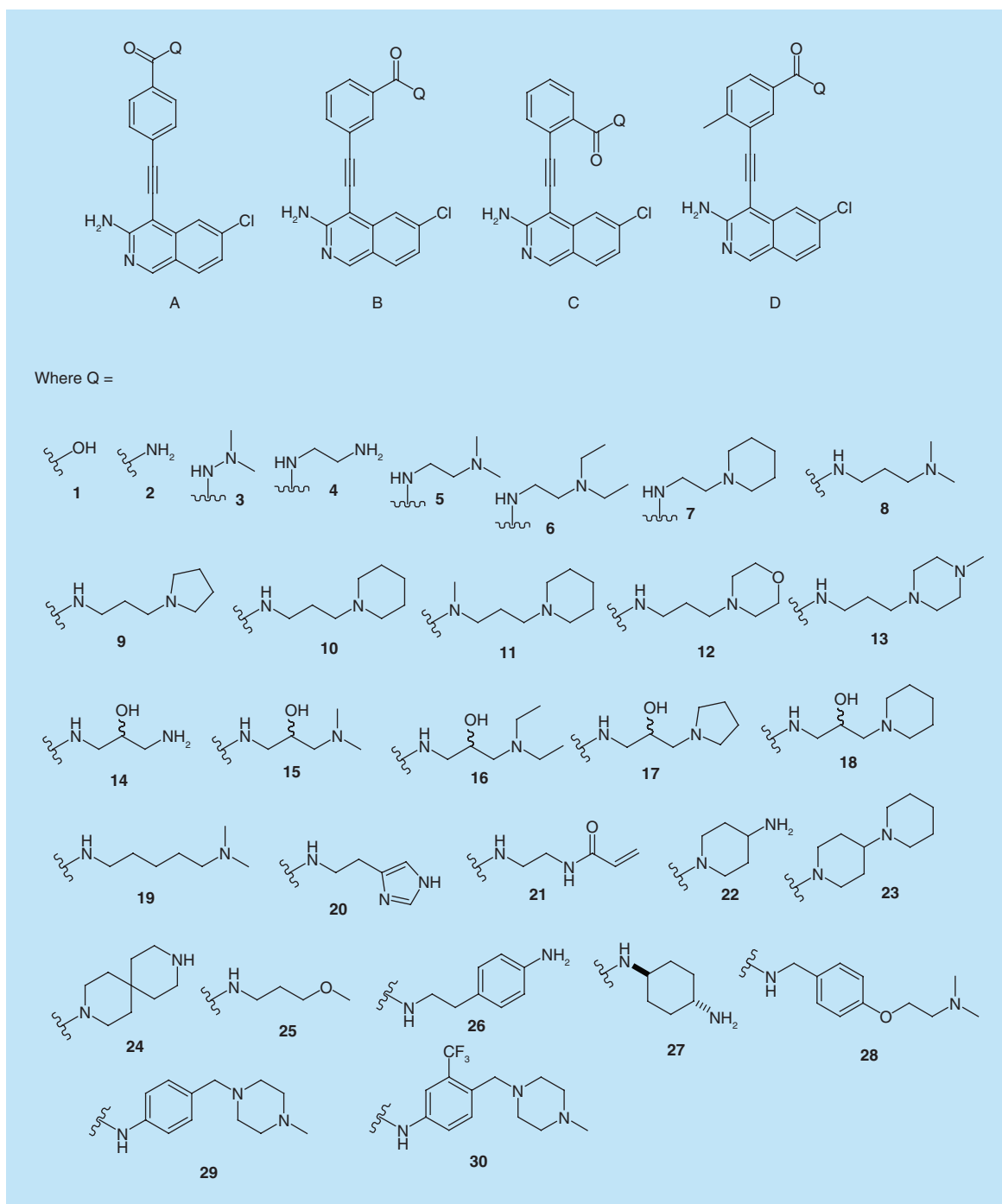
Figure 3. Representative examples of compounds synthesized. See Supplementary Information for a list of all compounds made.

FLT3 inhibitors, which could potentially be translated into anti-AML drugs [20].

A library of amino isoquinoline, quinoline or quinazoline compounds was synthesized via Sonogashira coupling (Figure 2). Four types of benzamide analogs of 3-aminoisoquinolines were first prepared (Figure 3): amide is *para* to the alkyne; amide is *meta*

to the alkyne; amide is *ortho* to the alkyne; and amide is *meta* to the alkyne and an additional methyl substituent next to the alkyne was also present. With a library of synthesized compounds, we proceeded to the investigation of antiproliferative activities against AML cell line, MV4–11 (a FLT3-driven cell line) and three other solid tumors (MCF7, breast; HCT116,

Table 1. Percent inhibition of cancer cell line proliferation in the presence of compounds (1 μM).

Compound	HSN210	HSN204	HSM1669	HSM1812	HSM137	HSM1673	HSM1683	HSM105	HSM1610	HSM1674	HSM1677	HSM1684	HSM1702	HSM1750	HSN145	HSN139	HSN135	HSM1773
Code	A3	A4	A5	A6	A7	A9	A10	A11	A12	A13	A14	A15	A16	A17	A20	A22	A23	A24
MV4-11	100	77	83	92	99	98	95	88	90	93	91	99	97	93	99	95	100	93
HCT116	6	21	46	17	60	28	10	40	39	14	15	33	38	20	35	32	21	24
HeLa	16	22	20	97	20	38	49	1	20	8	1	17	51	11	11	35	0	24
MCF-7	1	20	98	100	74	56	57	12	29	36	46	100	92	17	6	60	35	43

Table 2. Percent inhibition of cancer cell line proliferation in the presence of compounds (1 μM).

Compound	HSM1692	HSN136	HSN129	HSN99	HSN161	HSM1661	HSM1688	HSM1751	HSM1717	HSN189	HSN157	HSN174	HSN247	HSN248	HSN178	HSN185	HSN315	HSN286
Code	A25	A26	A27	A28	A29	B8	B15	B16	B17	B20	B22	C10	D6	D7	D15	D16	D28	D30
MV-4-11	81	86	99	97	90	74	97	95	91	96	85	82	58	98	98	97	83	99
HCT116	29	38	29	20	0	43	18	7	32	12	28	33	54	76	97	67	66	100
HeLa	22	0	22	6	1	64	70	18	23	33	10	2	100	100	100	99	100	100
MCF-7	22	33	37	42	96	33	91	49	47	21	40	76	100	100	100	100	100	100

colon and HeLa) (Tables 1 & 2, & Figure 4). From these cell proliferation studies, MV4–11 appeared to be more sensitive to the compounds than the other cell lines (Table 1 & 2). At 1 μM, most of the compounds could inhibit MV4–11 significantly. To identify group of compounds potently inhibiting cancer cell proliferation, we used a lower concentration of compounds (100 nM) to screen against MV4–11 (Figure 4). From these experiments, we selected potent amide compounds A7, A10, A15, A16, A18, A20, B15 and D30 (as indicated by ***, Figure 4). At 100 nM, these selected compounds inhibited MV4–11 at similar levels to midostaurin, a pan kinase inhibitor that recently completely a Phase III clinical trials (Figure 4). Typically amides that contain basic amines are included in compound libraries to improve aqueous solubility but it appears that the presence of a basic amine in the side chain of the compounds also facilitated the actual inhibition of MV4–11 proliferation. For example, compounds A1, A2, A21 and A25, which did not have a basic amine side chain, were inactive against MV4–11 whereas many of the other compounds containing a basic amine chain were active against MV4–11. We currently do not have an explanation for this observation and future structural work, beyond the scope of this report, could shed more light on the role of the basic amine. Stability of the active compounds, in the presence of mouse liver microsomes revealed that compounds with the D substitution pattern (such as D30) preformed much better in the liver microsomal stability assay compared with the other analogs.

Compound D30 contains 1-methyl-4-(2-[trifluoromethyl]benzyl)piperazine group, which is found in many kinase inhibitors, including ponatinib [21,22]. Ponatinib, which is used to treat imatinib-resistant CML, has been shown to inhibit FLT3-driven AML [21,22]. Unfortunately, ponatinib causes adverse cardiovascular effects [23] and it is now given with a black box warning and as a last resort drug against CML [24]. In 2014, it was withdrawn from the US market due to blood clotting and cardiovascular adverse effects [24] and therefore it might not be an ideal drug to advance for AML treatment (especially for elderly patients). We were therefore interested in exploring further analogs of D30 to investigate if the kinase selectivity of compounds containing the 1-methyl-4-(2-[trifluoromethyl]benzyl)piperazine group could be modulated via a judicious substitution on the isoquinoline core or use of isoquinoline isomers. Compounds HSN352, HSN353, HSN334, HSN356, HSN285 and HSN325 (containing 3-aminoisoquinoline, 1-aminoisoquinoline, 2-aminoquinoline and 2-aminoquinazoline, Figure 5) were prepared via the Sonogashira coupling. With these compounds in hand, we determined
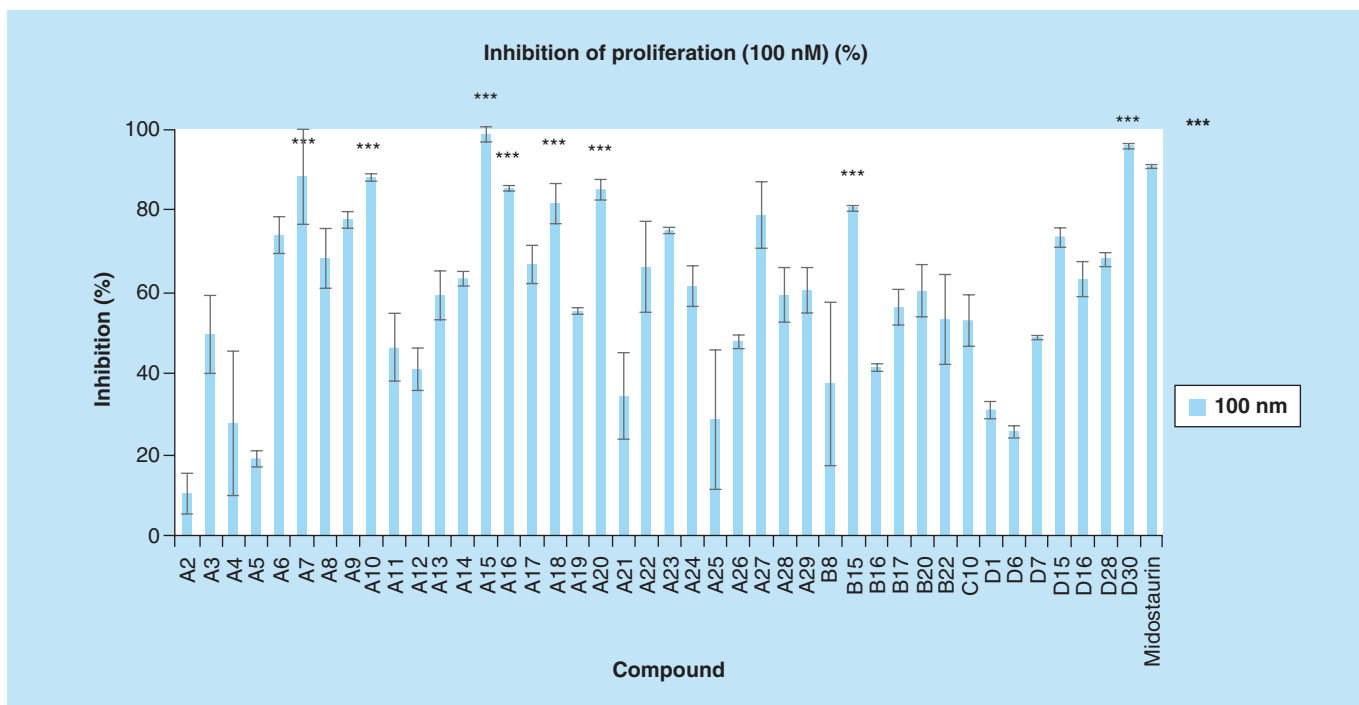
Figure 4. Percentage inhibition of proliferation in MV4–11 cell line with various analogs (100 nM). See Table 3 for IC_{50} values for selected compounds: D7, D15, D6, A15, D28, D30 and midostaurin.

the proliferation inhibition (IC_{50}) against MV4–11 and MOLM-14 (FLT3-driven AML cell lines) (Table 3).

HSN286 had an IC_{50} against MV4–11 and MOLM-14 of 0.5 and 0.7 nM, respectively (Table 3). The 1-aminoisoquinoline analogs, HSN334 and HSN356, were also potent proliferation inhibitors of MV4–11

and MOLM-14. The degrees of AML proliferation inhibition by the isoquinoline compounds were similar to (or even slightly better than) midostaurin (Table 3). In general, there was a good correlation between the percentage inhibition of FLT3 enzymatic reaction (obtained as percentage inhibition at 500 nM

| Table 3. IC_{50} (proliferation) and FMS-like tyrosine kinase 3 inhibition of activity.			
Compound	**% inhibition of FLT3 activity (500 nM)**	**IC_{50} (nM)**	
		MV4–11	**MOLM14**
HSN248 (D7)	73%	181 ± 6.5	180 ± 7.8
HSN178 (D15)	66%	144 ± 2.6	187 ± 5.5
HSN247 (D6)	74%	154 ± 7.1	129 ± 8.2
HSM1702 (A15)	66%	71 ± 1.1	102 ± 11
HSN315 (D28)	32%	549 ± 7.4	523 ± 6.4
HSN285	28%	721 ± 5.8	415 ± 6.9
HSN286 (D30)	97%	0.49 ± 0.02	0.72 ± 0.02
HSN325	33%	135 ± 5.87	456 ± 15
HSN334	99%	1.38 ± 0.1	1.61 ± 0.02
HSN353	94%	3.45 ± 0.04	1.97 ± 0.1
HSN352	93%	3.09 ± 0.1	3.01 ± 0.1
HSN356	98%	0.42 ± 0.02	0.62 ± 0.02
Midostaurin	98%[†]	18.5 ± 2.5	7.37 ± 0.1
[†]% FLT3 inhibition at 412 nM. FLT3: FMS-like tyrosine kinase 3.			
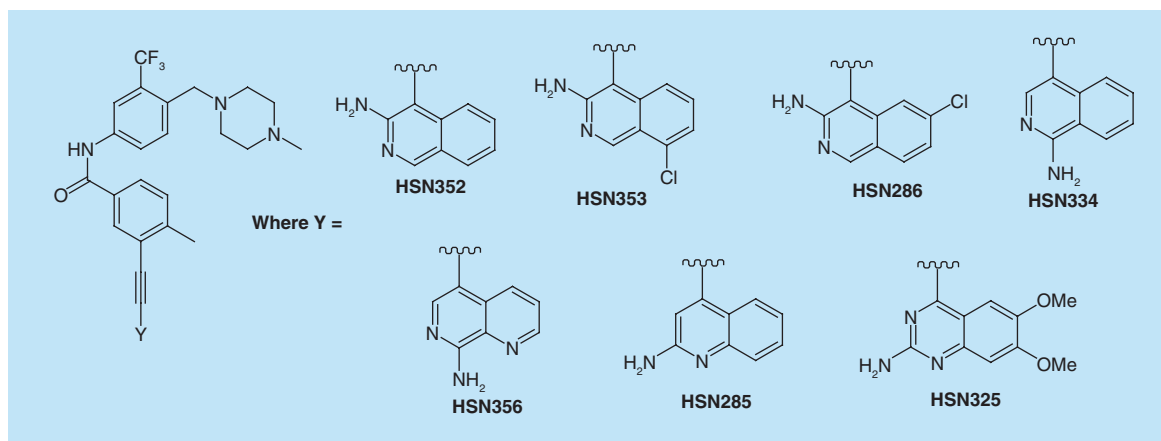
Figure 5. D30 analogs that were synthesized to investigate the influence of the quinoline/quinazoline/isoquinoline core on anticancer activity and kinase inhibition.

compound, Reaction Biology, PA, USA) and the inhibition of AML cell lines MV4–11 and MOLM-14 proliferation. Not all of the aminoquinoline compounds were potent inhibitors of the AML cell lines proliferation. For example, HSN248, HSN178, HSN247, MXC1702 and HSN315 that also contained the 2-aminoquinoline core were only moderate inhibitors of AML proliferation or FLT3 enzymatic activity. However, the 1-methyl-4-(2-[trifluoromethyl]benzyl) piperazine moiety (Figure 5) is not the sole determinant of FLT3 inhibition. The 2-aminoquinolines and quinazoline analogs, HSN285 and HSN325, both contain this moiety but they were neither potent inhibitors of FLT3 nor active against MV4–11 or MOLM-14 cell lines. Therefore, it appears that the potencies of these

compounds are due to the combined or synergistic effects of the 1-methyl-4-(2-[trifluoromethyl]benzyl)piperazine moiety (found in ponatinib) and the aminoisoquinoline moiety.

To test for selectivity, HSN286 was tested against various other cancer lines as well as a normal cell line (MRC5, lung fibroblast cell line), Figure 6. When tested against Kasumi-1, a non-FLT3 AML line, the IC_{50} of HSN286 was determined to be over 10,000 nM. The IC_{50} values for compound HSN286 against HL60, an acute promyelocytic leukemia cell line and THP1 (another non-FLT3-driven AML line) were 2156 and 2315 nM, respectively (i.e., more than 2000× less active against these cell lines when compared with FLT3-driven cell lines MV4–11 or MOLM-14). In
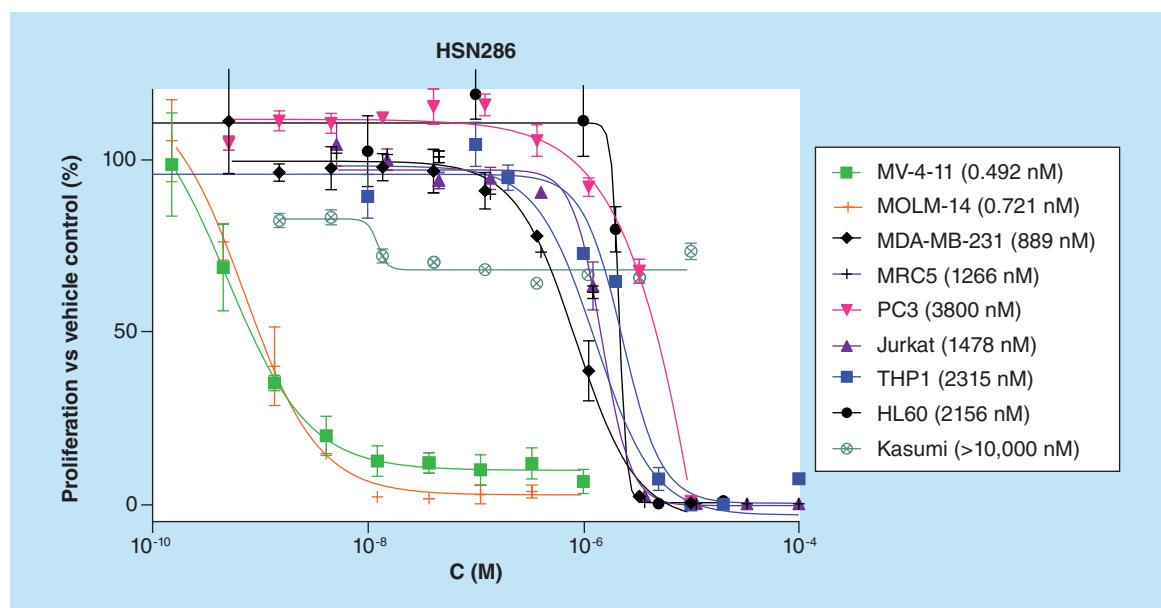

Figure 6. HSN286 (D30) is active against FMS-like tyrosine kinase 3 driven MV4–11 and MOLM-14 but not other cancer cell lines and normal cell line, MRC5.
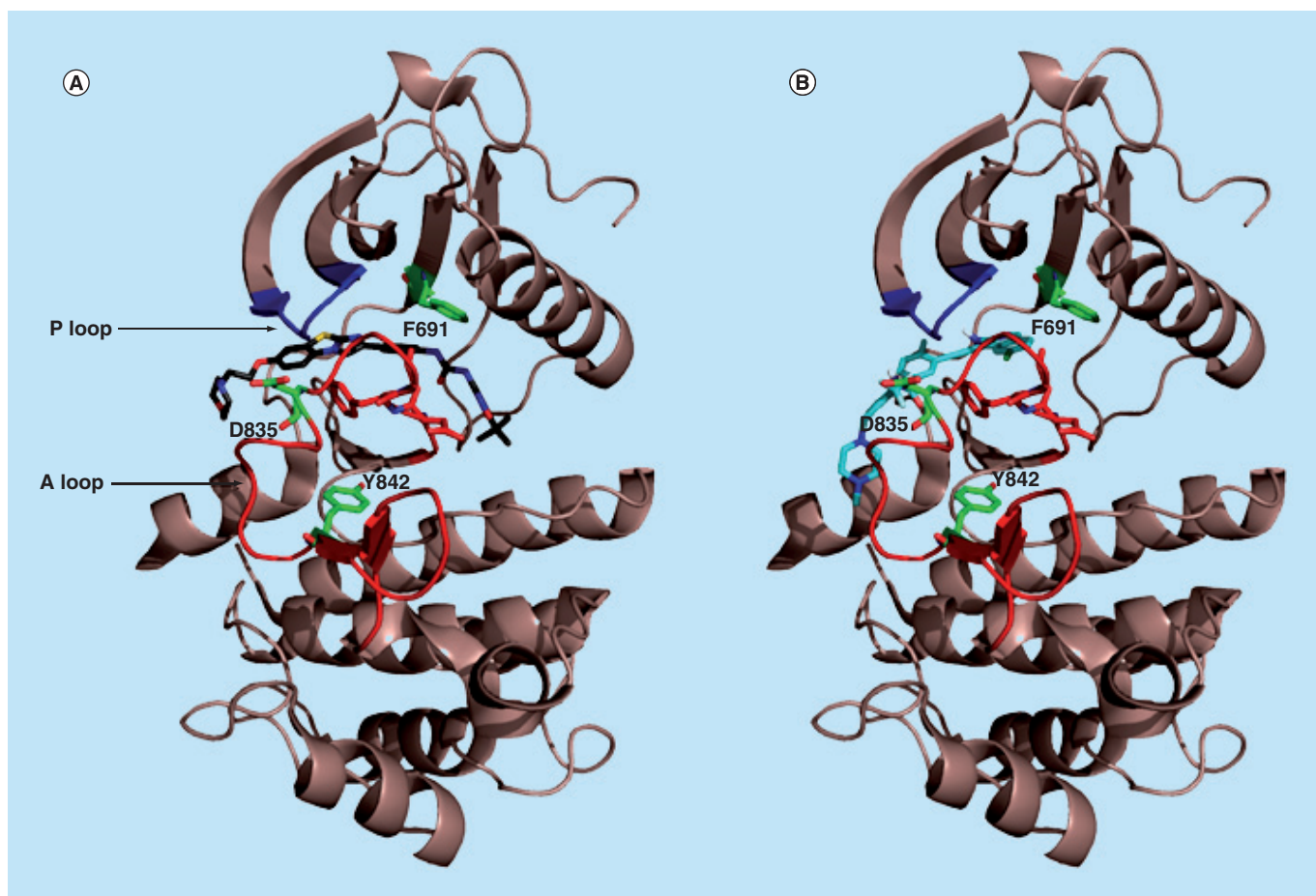
Figure 7. Quizartinib and HSN286 bound to FLT3 kinase. (A) Docked quizartinib bound to the inactive form of wild-type FLT3;
(B) docked HSN286 bound to the inactive form of wild-type FLT3. PDB # 4xuf. Docking was done with AutoDock Vina [24]. The docked
quizartinib matched the ligand in the crystal structure of FLT3/quizartinib. Key residues D835, Y842 and F691 which when mutated
block binding of FLT3 inhibitors are shown as sticks with green carbons. The DFG motif is represented as residues with red carbons.
The A loop and P loop (ATP binding) are colored as red and blue, respectively.
FLT3: FMS-like tyrosine kinase 3.

addition, the IC_{50} of compound HSN286 against MRC5 is 1266 nM (over 1000× less compared with IC_{50} for MV4–11 or MOLM-14). It therefore appears that HSN286 is selective (at least among the cell lines tested) for FLT3-driven leukemia.

Docking [25] of HSN 286, 334, 356 and 248 to the inactive conformer of FLT3 (a crystal structure of the potent FLT3 inhibitor, quizartinib, was solved in complex with FLT3, PDB # 4xuf) revealed that the compounds bind to both the ATP-binding site and neighboring site (Figure 7). The binding of the compounds partly overlaps with the quizartinib-binding site, but there are some differences in the binding modes (Figures 8 & 9).

Although the current drugs on the market or in clinical trials are relatively successful in killing the AML leukemia cells, they currently are unable to deal with secondary mutations that occur after TKI treatment, such as FLT3 (D385V) [26]. We investigated the

binding of HSN286, HSN336, HSN334, HSN248 and HSN247 with a few FLT3 secondary mutations (Table 4). Interestingly, HSN356 could bind to FLT3 (D835V) with a K_d of 19 nM, whereas a similar compound, HSN334, was a poor binder of FLT3 (D835V) with a K_d of 120 nM (Table 4).

Most kinase inhibitors that have found clinical success have been multikinase inhibitors. For example, sorafenib, used to treat kidney cancer, hepatocellular carcinoma and radioiodine-resistant thyroid cancers, also inhibits several kinases, such as RAF, MEK, ERK, VEGFR, PDGFR, FLT3, c-KIT, FGFR-1 and DDR2 [26]. Dasatinib, another multikinase inhibitor is a first-line treatment for CML and Ph⁺ ALL, inhibits a multitude of kinases [27]. This includes BCR-ABL, YES, EPHA8, c-KIT, SRC, LCK, DDR2, FRK, FYN, ARG, BTK and HCK [26]. Despite the promiscuity of these multikinase inhibitors, safe and tolerable doses have been found for cancer treatment. The successes

Table 4. K_d for isoquinoline analogs binding to FLT3, FLT3-ITD and FLT3 (D835V), determined via DiscoverX K_d Elect service.					
	HSN286	**HSN356**	**HSN334**	**HSN178**	**HSN248**
FLT3	6.5 nM	1.3 nM	5.1 nM	53 nM	47 nM
FLT 3 (ITD)	27.6 nM	6.7 nM	19 nM	450 nM	330 nM
FLT3 (D835V)	120 nM	19 nM	120 nM	ND	ND
FLT3: FMS-like tyrosine kinase 3; ITD: Internaltandem duplication; ND: Not determined.					

of these multikinase inhibitors could be derived, in part, from the simultaneous inhibition of different kinase axes. Therefore, we investigated if HSN286 and its analogs also inhibited other cancer-related kinases. In addition to FLT3, the Src-family kinases (such as BLK, FGR, FYN, HCK, LYN, SRC and YES) have been shown to play critical roles in leukemia [28–31]. Lopez *et al.* recently demonstrated that CDK6 over-expression in FTL3-ITD positive AML is achieved via the Src-family kinase, HCK [32]. HCK is expressed more in human primary leukemic stem cells than in normal human hematopoietic stem cells. A study showed that when HCK is targeted with small molecules, drug resistance is reduced [29]. Other protein kinases, such as SYK [33], BRAF, p38 (p38MAPK) [34], PDGFRα/β [35], FGFR1 [36], RET [37], FLT4 [38] and Tie2 [39] have also been linked to leukemia. All these data further strengthen the consensus in the field that leukemia is a heterogeneous disease and hence targeting the aforementioned multiple kinase pathways

could lead to a better outcome [40]. Therefore, we wanted to test if HSN286 and analogs were also targeting kinases that play critical roles in AML. The kinase screening services Reaction Biology and DiscoverX were used to characterize the inhibition of kinase activity (enzymatic activity in the presence of 500 nM of compounds, Supplementary Information S2). HSN286 and analogs potently inhibit FLT3 and the Src-family kinases but not other kinases, such as Aurora A, CDK6 or PIK3Ca (Table 5 & Supplementary Information). The inhibition of the Src-kinase family could be important clinically because these kinases are downstream of FLT3. In the event of FLT3 mutation, the inhibition of the Src-family kinases could still lead to proliferation inhibition [41].

In addition to the binding assays (Table 5), IC_{50} values for the inhibition of FLT3 kinase enzymatic activity by midostaurin, HSN286, 334 and 356 were determined. All of the four compounds could inhibit FLT3 with low nanomolar values, although midostaurin and
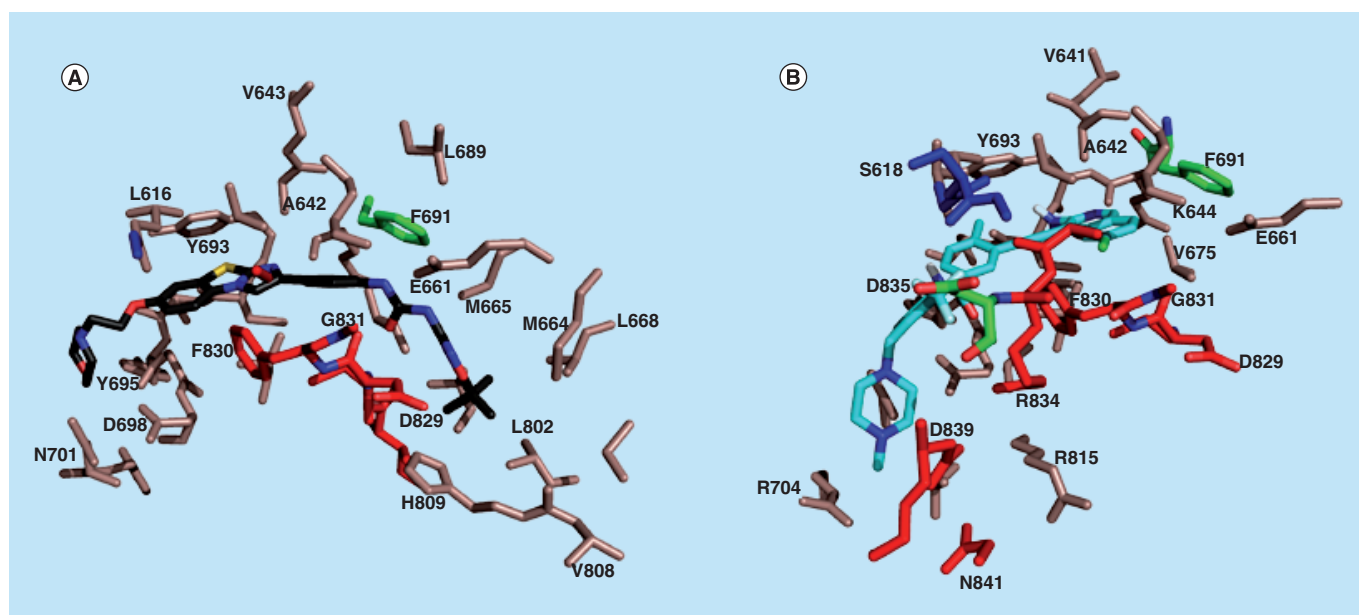

Figure 8. FLT3 residues that are near the bound ligands. (A) FLT3 residues that are within 6 Å of quizartinib in the docked structure; **(B)** FLT3 residues that are within 6 Å of HSN286 in the docked structure. The residues on the A loop including the DFG motif are shown as red while the P loop residues are colored blue.
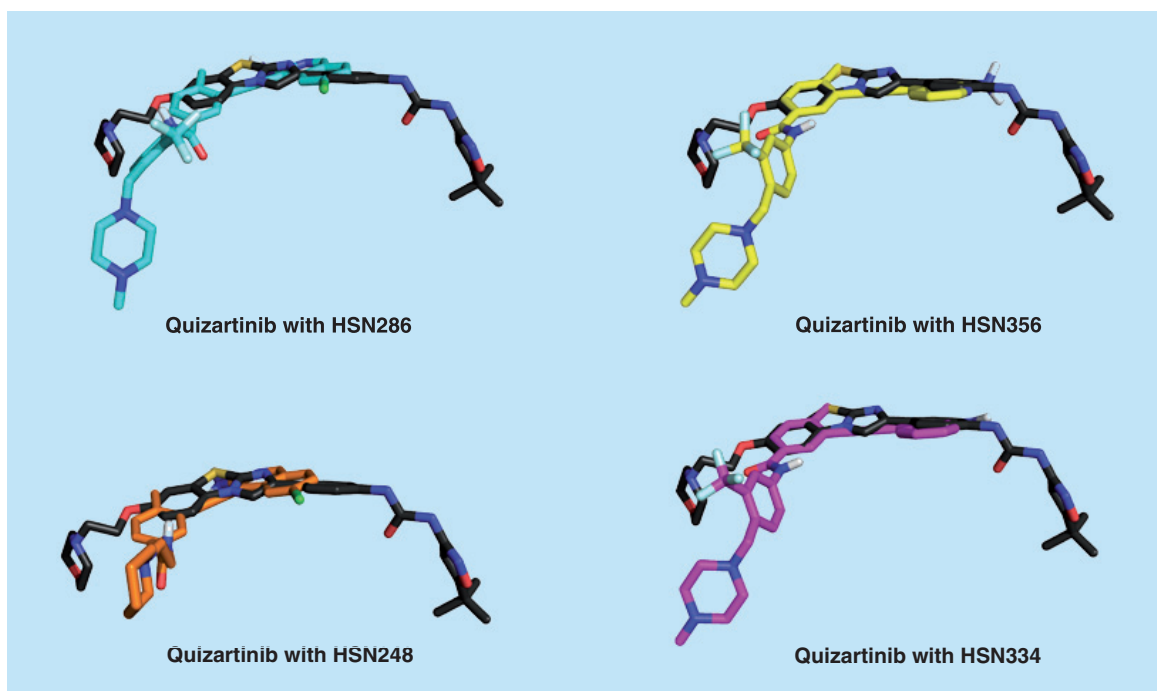FLT3: FMS-like tyrosine kinase 3.

Figure 9. Overlays of FMS-like tyrosine kinase 3 binding mode of quizartinib and HSN analogs.

HSN356 were better than HSN286 and HSN334 (Figure 10). Midostaurin and HSN356 inhibited FLT3 and FLT3 ITD with single digit IC_{50} values. However in the case of Src kinase, HSN286 was the most potent (IC_{50} of 4.5 nM). HSN356 is also an effective inhibitor of Src kinase with an IC_{50} of 14.9 nM.

To determine if the phosphorylation of FLT3, SRC kinase and downstream effectors or kinases (such as STAT3 [42], STAT5 [43] and p-38 [44]) could be affected by the compounds, we performed western blot analysis of total protein obtained from MV4–11 treated with HSN286 for FLT3/phosphorylated FLT3, STAT5/ phosphorylated STAT5, STAT3/phosphorylated STAT3 as well as SRC kinase/phosphorylated SRC and p-38/ phosphorylated p-38 to ascertain that one could indeed target FLT3 and associated signaling axis in cells (target engagement or confirmation, Figure 11). In line with the *in vitro* kinase inhibition data, the phosphorylation of FLT3, STAT5, STAT3 and p-38 could be inhibited by HSN286 (Figure 11). The level of SRC kinase increased over time (amount at the 48-h time point is greater than at the 6- and 24-h time points). Unfortunately, the bands for the phosphorylated SRC kinase are blurred despite repeated attempts to get clearer bands. Despite the technical challenge with the phospho-SRC western, it is conclusive that at the 48-h time point, the level of unphosphorylated SRC kinase increases as the compound HSN286 is added (compare the band corresponding to vehicle [V] to bands when cells were treated with 9 and 45 nM HSN286 in

Figure 11). From the above western analyses, we confirm that the proliferation inhibition of MV4–11 is due to the inhibition of FLT3 signaling axis.

Conclusion

We have developed novel FLT3 inhibitors that can inhibit the problematic D835 secondary mutants.

Table 5. K_d (nM) determined via DiscoverX K_d Elect service.

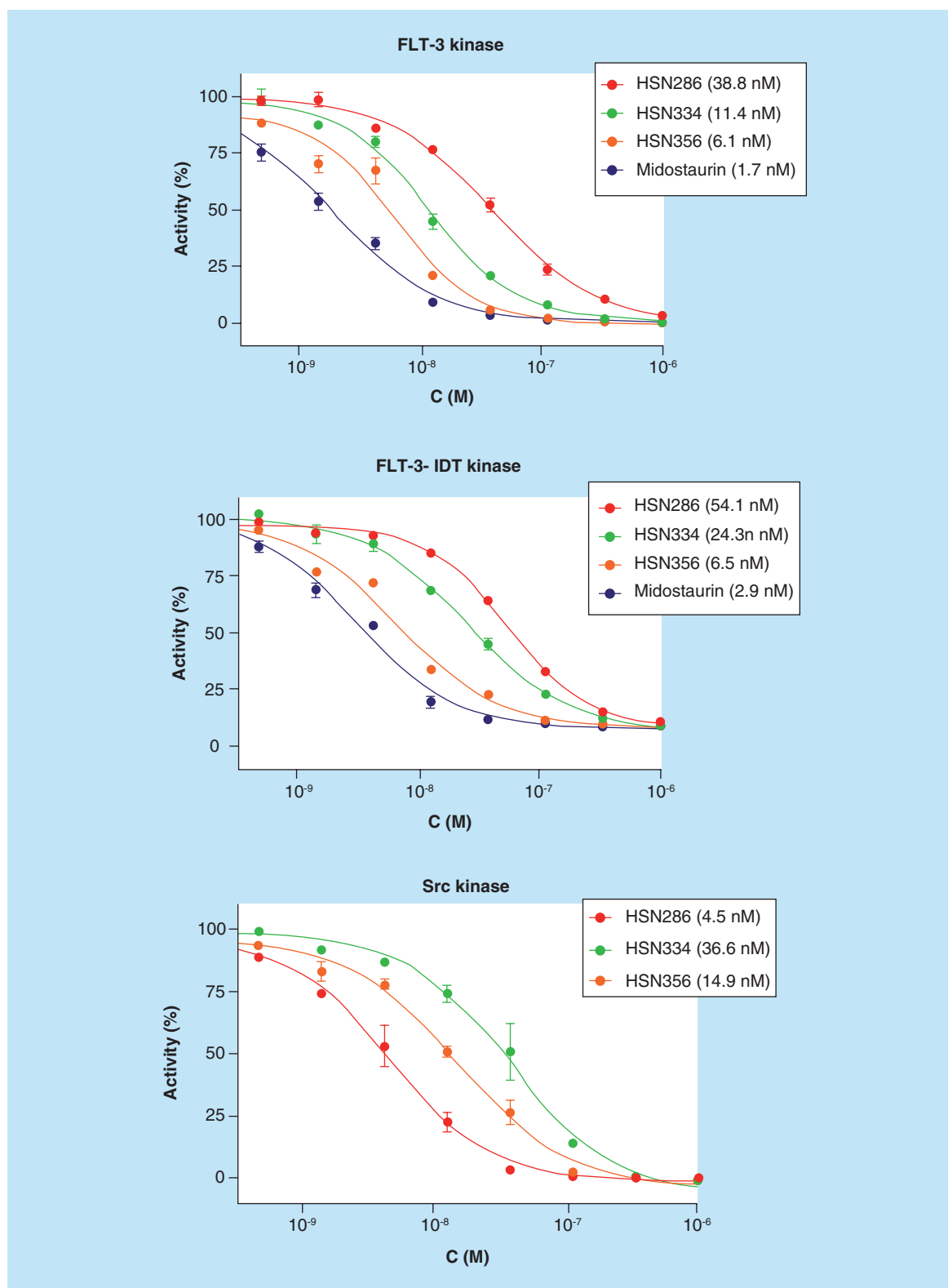| Kinase	HSN286	HSN356
AURKA	>30,000	5100
BLK	3.9	1.2
CDK6	28,000	ND
CDK9	2300	ND
FAK	5100	4700
FGR	7	7.9
FLT3	7.2	1.3
FYN	28	38
HCK	3.6	2.5
KIT	42	7.2
LYN	11	5.4
PIK3CA	26,000	>30,000
PIM1	11,000	19,000
PLK1	>30,000	9600
SRC	16	15

ND: Not determined.

Figure 10. Enzymatic inhibition of FLT3, FLT3 ITD and Src kinases by select compounds.

Specifically, 4-alkyne substituted aminoisoquinolines (readily obtained via Sonogashira coupling) but not the related quinolines, were potent FLT3 and SRC-kinase inhibitors. Extensive SAR studies revealed that the FLT3 inhibition profiles and antiproliferative activities against FLT3-driven cancer cell lines,

MV4–11 and MOLM-14 were dictated by the substitution pattern and nature of benzamide. These SAR studies have led to the identification of 3-amino and 1-aminoisoquinoline benzamides, compounds D30 (HSN286), HSN334 and HSN356 as potent FLT3 kinase inhibitors. Some of these novel kinase inhibitors also inhibit the proliferation of FLT3-driven AML cell lines at concentrations as low as 500 pM. In addition to FLT3, we identified compounds that also inhibit the Src-family kinases and FGFR kinases (Supplementary Information). We also show that one could combine different isoquinolines with 1-methyl-4-(2-[trifluoromethyl]benzyl)piperazine group (a privileged moiety in kinase inhibitors) to develop analogs that have different kinase selectivities (a kind of 'plug-and-play' strategy). This work has unveiled a new class of aminoisoquinoline benzamide kinase inhibitors, which have a high potential for clinical translation. Future studies that investigate the efficacy, pharmacokinetics/pharmacodynamics (PK/PD) of the selected potent inhibitors and its analogs on animal cancer models will be done to assess their potential as clinical lead compounds.

Future perspective

About 30% of AML patients carry mutated FLT3 kinase (such as FLT3-ITD) and hence efforts have been directed at the development of FLT3 inhibitors. Despite the availability of potent and selective FLT3 inhibitors, such as quizartinib, a complete cure for FLT3-driven AML has not been found [12]. Secondary mutations in FLT3, such as D835, cause resistance to FLT3-directed therapies. In addition, compensatory activated pathways account for resistance to FLT3 inhibitors. Perhaps successful AML therapies that will achieve complete cure would have to inhibit FLT3-ITD and secondary mutations, such as D835V/H/Y and F691I as well as downstream or compensatory signaling axes. Indeed, FLT3 inhibitors that also inhibit other pathways are beginning to emerge and such compounds could achieve the elusive complete cure for FLT3-driven AML, especially for older patients [45,46].

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/fmc-2017-0067
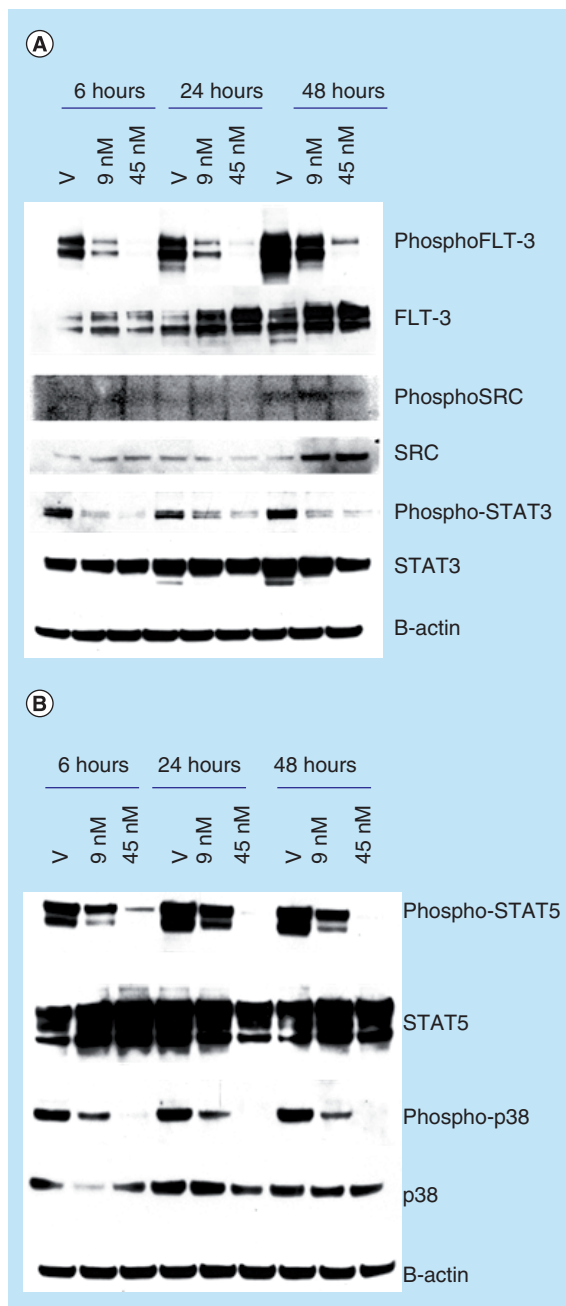
Figure 11. Western blot analysis after treating MV4–11 with HSN286. (A) Phospho-FLT3/FLT3, phosphoSRC/SRC and phospho-STAT3/STAT3; **(B)** phospho-STAT5/STAT5 and phospho-p-38/p-38. Cells were treated with DMSO vehicle (V), HSN286 (9 and 45 nM) for 6, 24 and 48 h. Western blot with antirabbit or antimouse antibody. Scanned images were analyzed using image J software. FLT3: FMS-like tyrosine kinase 3.

> **Summary points**
>
> - Mutated or overexpressed FLT3, drives about 30% of reported acute myeloid leukemia (AML).
> - Many FLT3 inhibitors have shown durable clinical responses but a complete cure of AML with FLT3 inhibitors remains elusive.
> - The lead aminoisoquinolines, HSN286, -334 and -356 potently inhibited FLT3-driven AML cell lines, MV4–11 and MOLM-14 (single-digit or subnanomolar IC_{50} values).
> - Some of the tested aminoisoquinolines inhibited kinases with secondary mutations, such as FLT3 (D835V), which have been traditionally difficult to inhibit with tyrosine kinase inhibitors.

References

Papers of special note have been highlighted as: • of interest;
•• of considerable interest

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