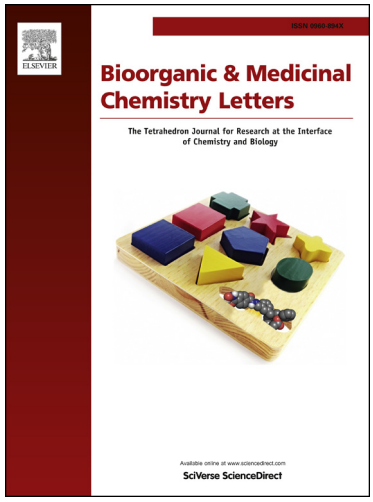

Synthesis and SAR studies of 5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine derivatives as potent inhibitors of Bloom Helicase

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Synthesis and SAR studies of 5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine derivatives as potent inhibitors of Bloom Helicase

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KEYWORDS. Bloom Helicase, Small molecule, inhibitor

ABBREVIATIONS: BLM, Bloom helicase; SAR, Structure Activity Relationship; HTS, High Throughput Screen; MIC, minimum inhibitory concentration; ADME, absorption, distribution, metabolism and excretion; MLM, mouse liver microsomes; NADPH, nicotinamide adenine dinucleotide phosphate; PBS, phosphate buffered saline; BS, Bloom syndrome; SCE, sister chromatid exchanges; HR, homologous recombination; DMF, dimethylformamide

Human cells utilize a variety of complex DNA repair mechanisms in order to combat constant mutagenic and cytotoxic threats from both exogenous and endogenous sources. The RecQ family of DNA helicases, which includes the Bloom helicase (BLM), plays an important function in DNA repair by unwinding complementary strands of duplex DNA as well as atypical DNA structures such as Holliday junctions. Mutations of the *BLM* gene can result in Bloom syndrome, an autosomal recessive disorder associated with cancer predisposition. BLM-deficient cells exhibit increased sensitivity to DNA damaging agents indicating that a selective BLM inhibitor could be useful in potentiating the anticancer activity of these agents. In this work, we describe the medicinal chemistry optimization of the "hit" molecule following a quantitative high-throughput screen of >355,000 compounds. These efforts lead to the identification of ML216 and related analogs, which possess sub-micromolar BLM inhibition and exhibit selectivity over related helicases. Moreover, these compounds demonstrated cellular activity by inducing sister chromatid exchanges, a hallmark of Bloom syndrome.

The human genome is constantly being threatened by potentially harmful DNA-damaging events. In order to maintain genome integrity, cells have developed various mechanisms for detecting DNA damage and coordinating its repair. A fundamental feature of DNA repair, as well as other cellular processes involving nucleic acids, is the transient separation or unwinding of the DNA double helix into complementary strands permitting access to genetic information. DNA helicases are a continually expanding group of enzymes that catalyze this strand separation reaction.[1] Indeed, helicases are predicted to constitute approximately 1% of the open reading frames in the human genome. One highly conserved family of DNA helicases, termed the RecQ helicases, plays an important function in genome preservation through diverse roles in DNA repair, recombination, and replication (for review, *see* [2]). The human genome encodes five RecQ helicases and genetic defects in at least three of these genes, *BLM*, *WRN*, and *RECQ4*, are responsible for distinct human disorders that cause premature aging and/or cancer predisposition, among other phenotypes (for review, *see* [3]). For example, Bloom syndrome (BS) is a rare autosomal recessive disorder originating from loss-of-function mutations in the *BLM* gene.[4] BS clinically exhibits a pleiotropic phenotype characterized by proportional dwarfism, sun-sensitive telangiectatic erythema, fertility defects, immunodeficiency, and shortened lifespan, which is typically cancer-related.[5] Cells from BS patients are characterized by an elevated level of genomic instability and a genome-wide increase in sister chromatid exchanges (SCE), which is a key feature used in the clinical diagnosis of the disorder.[6]

The *BLM* gene product is an ATP-dependent DNA helicase that translocates in the 3'–5' direction.[7] BLM helicase has been shown to resolve a wide variety of DNA structures, including 3′-tailed duplexes, bubble and splayed arm DNA structures, DNA displacement loops (D-loops), four-way Holliday junctions, and G-quadruplex structures.[8] In addition, BLM forms a multiprotein complex with RMI1, RMI2, and topoisomerase IIIα that functions in the dissolution

of double Holliday junctions,[9] which are prominent intermediates in the homologous recombination (HR) repair pathway.[10] The involvement of BLM in double-strand break repair is corroborated by its interaction with RAD51 recombinase, which is the essential enzyme in HR that catalyzes homology-dependent strand invasion.[11] Current research is also establishing the role of BLM in telomere maintenance[12] as well as the processing and re-initiation of stalled replication forks.[13]

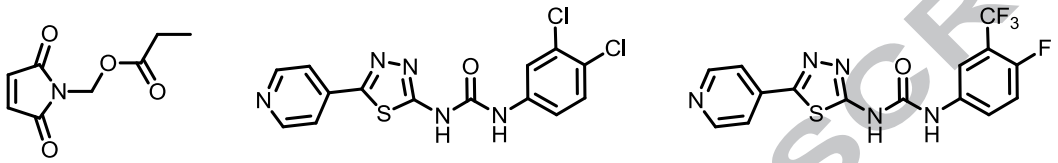
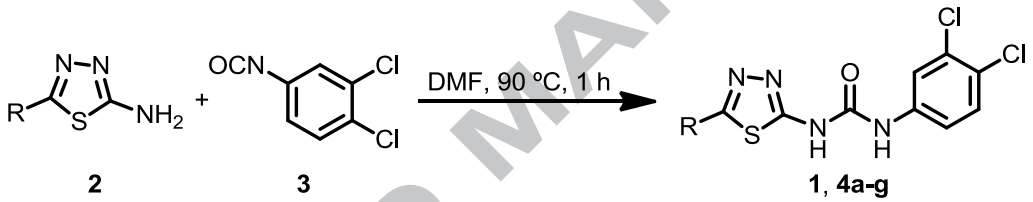


Figure 1. Structures of previously identified RecQ helicase inhibitors. Recently published WRN inhibitor, *left*, and BLM inhibitors (*middle*, HTS hit and *right*, optimized probe).

Prior reports have revealed only non-specific, weakly active RecQ helicase inhibitors. For example, several clinically used DNA-binding compounds have been described as non-specific inhibitors of both BLM and WRN-catalyzed DNA unwinding activity.[14] A recent screen of the NCI diversity set identified NSC19630 (Figure 1) as a small molecule inhibitor of WRN helicase.[15] Although this maleimide-containing compound potentially suffers from promiscuity given the known reactivity of such moities with cysteine residues, it does highlight the growing interest in the helicase field.[16] More recently, we described the discovery and biological activity of **ML216** (Figure 1), a novel small molecule inhibitor of BLM helicase. **ML216** was found to possess potent (1-3 μM) inhibition of the DNA unwinding activity of BLM, induce sister chromatid exchanges, and demonstrate selective antiproliferative activity in BLM-positive cells.[17] Herein, we detail the medicinal chemistry efforts that led to the nomination of **ML216** as a chemical probe and provide selectivity information and *in vitro* ADME data for additional analogs.

Before initiating an extensive medicinal chemistry effort, we first sought to resynthesize and confirm the activity of the HTS hit molecule MLS000559245 (**1**) (Figure 1). Pure solid compound was prepared in one-step by heating (90 ºC) commercially available starting materials, 2-Amino-5-(4-pyridinyl)-1,3,4-thiadiazole and 3,4-dichlorophenyl isocyanate, in DMF.[17] Subsequent testing of this resynthesized compound (now denoted as **1**) in a conventional gel-based DNA unwinding assay for BLM inhibitory activity confirmed the HTS activity with an IC_{50} value of 1.4 µM (Table 1). The general synthetic procedure for the preparation of analogs **4a-g** is outlined in Scheme 1. A variety of thiadiazole-2-amines (**2a-h**) are commercially available and thus rapid SAR could be obtained for this region of the molecule using the previously described method.



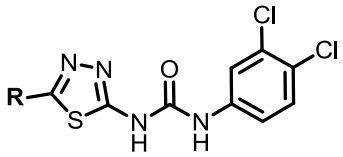
2a: R = 4-pyridine
2b: R = phenyl
2c: R = 2-pyridine
2d: R = 3-pyridine
2e: R = 4-amino-phenyl
2f: R = piperazine
2g: R = N-Me-piperazine
2h: R = 4-amino-piperidine

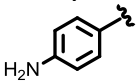
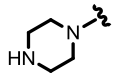
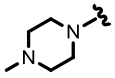
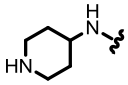
Scheme 1. Reagent and conditions: Method A: DMF, 90 °C, 1h (See supplemental information for details)

Our first area of SAR exploration involved modification of the 4-pyridine moiety on the western region of the molecule (Table 1). These compounds were synthesized via reaction of the requisite thiadiazole-2-amine derivative with 3,4-dichlorophenylisocyante as shown in Scheme 1. Changing the pyridine group to a phenyl (**4a**) and changing the position of the pyridine nitrogen from the 4-position to the 2-position (**4b**) led to inactivity. Interestingly, the 3-pyridine (**4c**) had comparable activity to the lead compound with an IC_{50} value of 3.5 µM. As

anticipated given the inactivity of phenyl analogue **4a**, the cyclohexane derivative (**4d**) was also inactive. These data suggest a preference for a basic nitrogen group in this region of the molecule; however, all attempts to mimic this interaction with other nitrogen containing heterocycles were largely unsuccessful (**4e-h**). Only the 4-aminophenyl derivative (**4e**) exhibited very modest inhibitory activity (IC_{50} = 49 μM).

Table 1. SAR of the 4-pyridine moiety (analogs **1, 4a-h**).

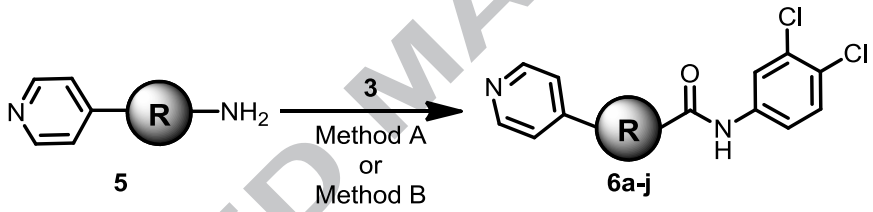


Compound	R	IC_{50} (μM)[a]
1		1.4
4a		inactive
4b		inactive
4c		3.5
4d		inactive
4e		49
4f		inactive
4g		inactive
4h		inactive

[a] Determined by gel-based BLM DNA unwinding assay.

Having established the importance of the 4-pyridine moiety on the western region of the molecule, we then turned our attention towards modification of the 1,3,4-thiadiazole core. Surprisingly, this region was also very intolerant to structural modification. The synthesis of these compounds was achieved through the reaction of the requisite amino derivative (**5a-j**) and

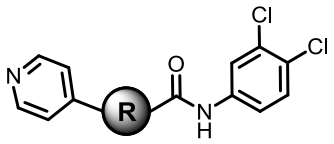
3,4-dichlorophenylisocyanate (**3**) as shown in Scheme 2. Amino derivatives (**5a-h**) were all commercially available; however, pyridine- (**5i**) and pyridimide- (**5j**) amines were prepared via a Suzuki coupling of 4-pyridine boronic acid with 6-chloropyridine-2-amine and 2-chloropyrimidine-4-amine, respectively. Changing the thiadiazole to the thiophene derivative (**6a**) resulted in a loss of activity. Interestingly, the 4-substituted thiazole (**6b**, IC_{50} = 28 μM) maintained some BLM inhibitory activity, yet the 5-substituted thiazole (**6c**) was completely inactive despite being more structurally similar to the original HTS hit. Replacement of the 1,3,4-thiadiazole for an 1,3,4-oxadiazole (**6d**) or other nitrogen containing heterocycles (**6e-h**) also resulted in loss of activity. Pyridine analog (**6i)** was the only core modification that was tolerated with an IC_{50} comparable to **1** of 4.0 μM, as even the related pyrimidine analog (**6j**) was inactive.

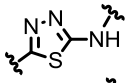
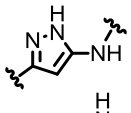
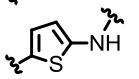
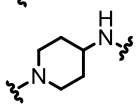
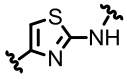
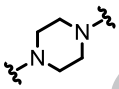
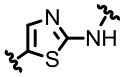
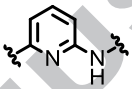
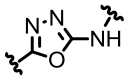
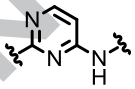
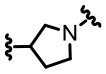


5a: R = thiophene
5b: R = 4-(thiazole-2-amine)
5c: R = 5-(thiazole-2-amine)
5d: R = 5-(1,3,4-oxadiazole-2-amine)
5e: R = 3-pyrrolidine
5f: R = 3-(*1H*-pyrazole-5-amine)
5g: R = piperidine-4-amine
5h: R = piperazine
5i: R = 6-(pyridine-2-amine); synthesized using conditions (b)
5j: R = 2-(pyridimide-4-amine); synthesized using conditions (b)

Scheme 2: Reagents and conditions (See supplemental data): Method A: DMF, 90 ^{o}C, 1h; Method B: analog **6i**: 6-chloropyridine-2-amine, **6j**: 2-chloropyridimide-4-amine; Na_2CO_3 (3.0 eq.), $Pd(PPh_3)_4$ (10 mol%), DME:H_2O (10:1), 150 ^{o}C, 1 h.

Table 2. SAR of the thiadiazole moiety (analogs **1, 6a-j**).

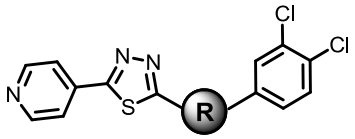


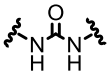
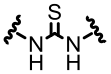
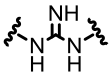
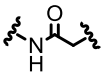
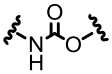
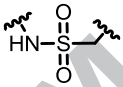
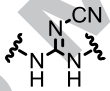
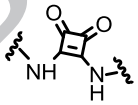
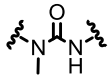
Compound	R	IC_{50} (μM)[a]	Compound	R	IC_{50} (μM)[a]
1		1.4	**6f**		inactive
6a		inactive	**6g**		inactive
6b		28.1	**6h**		inactive
6c		inactive	**6i**		4.0
6d		inactive	**6j**		inactive
6e		inactive			

[a] Determined by gel-based BLM DNA unwinding assay.

Next we explored the role of the urea moiety by replacement with comparable functional groups and known urea bioisosteres as shown in Table 3. The compounds were synthesized via a variety of methods with additional details provided in the Supporting Information. Converting the urea to a thiourea (**7a**) was tolerated (IC_{50} = 4.0 μM), however, given the comparable activity and that thioureas represent a potential toxicophore, additional analogs with this moiety were not pursued.[18] Replacement of the urea with a guanidine (**7b**), amide (**7c**), carbomate (**7d**) sulfonamide (**7e**) and cyanoguanidine (**7f**) all yielded weakly active or inactive molecules. Interestingly, squarate (**7g**) a known urea bioisostere, demonstrated comparable activity with an IC_{50} value of 4.3 μM, however the solubility of this compound was not improved over (**1**). Even mono-methylation of one of the urea nitrogens (**7h**) resulted in a complete loss of activity. Due to the lack of noticeable improvements in activity via modification at this particular part of the lead molecule, we continued our chemical efforts with the urea linker in place.

Table 3. SAR of the urea moiety (analogs **1, 7a-h**).



Compound	R	IC_{50} (μM)[a]
1		1.4
7a		4.0
7b		14.1
7c*		inactive
7d*		inactive
7e		inactive
7f**		inactive
7g		4.3
7h*		inactive

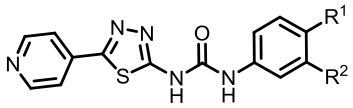
* 2-naphthalene instead of 3,4-Cl-phenyl
** 4-F,3-CF_3-phenyl instead of 3,4-Cl-phenyl

[a] Determined by gel-based BLM DNA unwinding assay.

Thus far, investigations of the initial compound (**1**), yielded tractable SAR with definite trends, but no analogs with improved potency were found and a general lack of tolerance for structural modifications was observed (*see* Tables 1-3). Our first experience with potency improvement came in the SAR studies of the eastern di-chlorophenyl moiety as shown in Table 4. These compounds were synthesized using the same method described in Scheme 1. Note that for

compounds in which the desired phenylisocyanate derivative was not commercially available, we synthesized it *in situ* via reaction of the requisite aniline with triphosgene.[19]

Table 4. SAR of the di-chlorophenyl moiety (analogs **1, 8-36**)



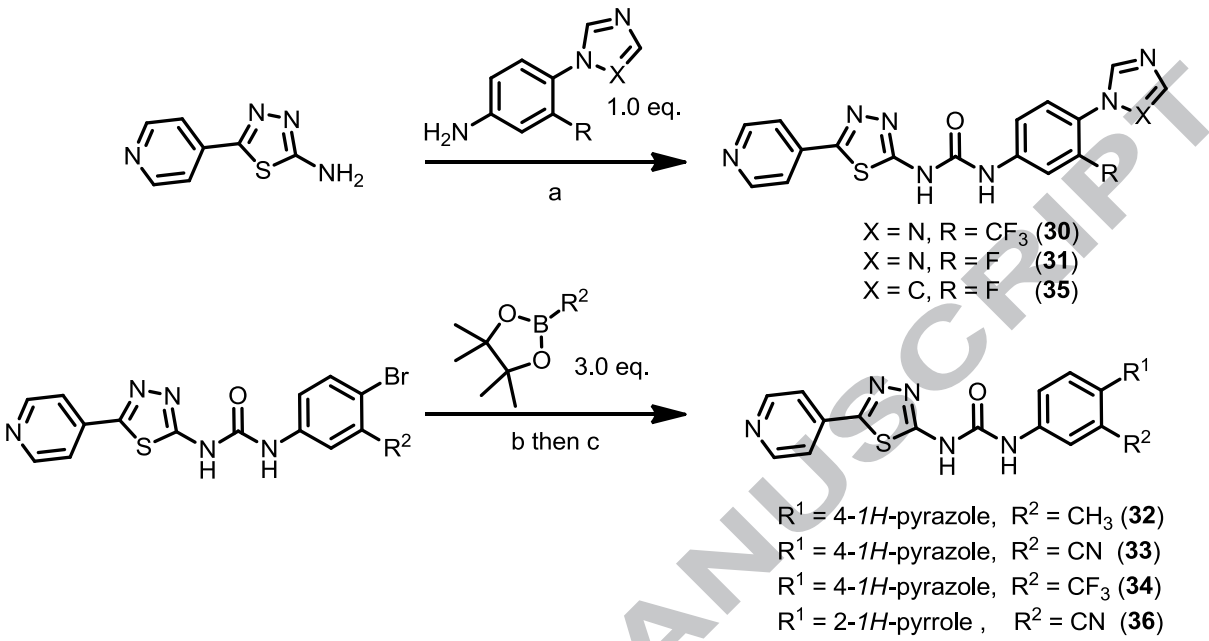
Compound	R^1	R^2	IC_{50} (μM)[a]	Compound	R^1	R^2	IC_{50} (μM)[a]
1	Cl	Cl	1.4	**25**	F	F	8.9
8	Cl	H	40	**26**	F	CH_3	15.8
9	H	H	inactive	**ML216**	F	CF_3	1.8
10	Cl	CN	0.10	**27**	F	H	14.1
11	Cl	CH_3	7.9	**28**	F	CN	0.3
12	Cl	CF_3	7.1	**29**	NH_2	CF_3	40
13	Cl	NO_2	1.3	**30**		CF_3	36
14	Cl	NH_2	11.2				
15	CN	Cl	1.6	**31**		F	14.1
16	CN	H	7.9				
17	H	CN	8.9	**32**		CH_3	16.8
18	CN	CH_3	3.2	**33**		CN	1.1
19	CN	CF_3	5.6				
20	Br	Br	2.1	**34**		CF_3	14.2
21	Br	H	12.6				
22	Br	CH_3	13.4	**35**		F	10.0
23	Br	CN	0.11				
24	CN	Br	0.91	**36**		CN	1.8

[a] Determined by gel-based BLM DNA unwinding assay.

We found that removal of one (**8**) or both (**9**) of the chloro atoms led to a drastic loss in activity. However, a noticeable improvement in potency (4-fold) was observed when the 3-Cl was replaced with a cyano group (**10**, IC_{50} = 0.1 μM). A similar trend was observed with the 3-CN, 4-Br-phenyl derivative (**23**, IC_{50} = 0.11 μM) and the 3-Br,4-CN analog (**24**) was also well tolerated with an IC_{50} value of 0.91 μM. Changing the initial bis-chlorophenyl ring to the isosteric 2-naphthalene moiety resulted in very little change in potency (data not shown) and

only added to the lipophilicity of the molecule so additional derivatives around the 2-napthalene were not explored. Replacing the chloro functionality with Br (**20**) or F (**25**) was tolerated, but did not result in improved potency with IC_{50} values of 5.0 and 8.9 μM, respectively. A variety of other aromatic substitutions were tried (entries **11-19**) and most were tolerated, but did not result in an increase in potency. Generally, electron-withdrawing groups at the 3-position [NO_2 (**13**), CN (**10**), Br (**20**)] were preferred over electron-donating groups [CH_3 (**11**), NH_2 (**14**)]. Despite the fact that introduction of the fluoro group appeared to result in a less desirable gel assay IC_{50} when compared to Br or Cl containing analogs, the 3-CF_3, 4-F-phenyl analog (**ML216**) demonstrated good activity that was reproducible in orthogonal bioassays and had other favorable pharamacokinetic properties. For example, while we were able to identify compounds more potent than **ML216** (e.g. analogs **10, 23, 24**), these compounds had noticeably diminished aqueous/DMSO solubility. Accordingly, we declared **ML216** as our chemical probe for the Molecular Library program given the optimal balance of potency and physicochemical properties in relation to other analogs. Subsequent to these studies, we remained interested in further improving the physicochemical properties of **ML216**, and more specifically the aqueous solubility. Therefore, we sought to introduce nitrogen containing heterocycles in an effort to increase the basicity/polarity of the molecules.[20] Preliminary investigations towards this end revealed that the 4-position of the eastern phenyl group was more tolerant to these modifications (data not shown). The synthesis of these compounds was accomplished through the requisite 4-Br derivative via Suzuki coupling (for pyrazoles) or Cu-catalyzed coupling (triazoles and imidazoles) as shown in Scheme 3. Introduction of the triazole moiety (entries **30, 31**) was tolerated yet resulted in a significant loss of potency with IC_{50} values of 36 and 14.1 μM, respectively. Imidazole (entry **35**) and pyrrole (entry **36**) derivatives both had good activity, however we were particularly intrigued by the activity of the 3-CN, 4-pyrazole phenyl derivative

(**33**) which possessed comparable activity to the probe compound. Importantly, analog **33** has a much improved *C*Log P value compared to **ML216** of 2.0 vs. 3.8 as shown below in Table 6.



Scheme 3. Synthesis of heterocyclic derivatives at the 4-position. Reagent and conditions (See Supporting Information for details): (a) analogs **30** and **31**: aniline [X = N and R = CF_3 and F, respectively], analog **35**: aniline [X = C, R = F]; triphosgene (1.0 eq.), NEt_3 (3.0 eq.), CH_2Cl_2, rt, 3h, then concentrate and add 5-(pyridin-4-yl)-1,3,4-thiadiazol-2-amine (1.0 eq.), DMF, heat to 90 ^oC, 1h; (b) analog **32-34**: [R^1 = CH_3 (**32**), CN (**33**), CF_3 (**34**); R^2 = 4-(Boc-1H-pyrazole)]; analog **36**: [R^1 = CN, R^2 = 2-(*1*H-pyrrole)] K_3PO_4 (2.5 eq.), $Pd(PPh_3)_4$ (10 mol %), Dioxane/DMSO/water (1/0.3/0.2), 100 ^oC, 3 hr. For analogs (**32**-**34**): (c) TFA, CH_2Cl_2, rt.

After completing our initial SAR exploration of the HTS "hit" molecule (**1**), we aimed to determine selectivity of top compounds against related RecQ helicases. As summarized in Table 5, several compounds demonstrated both improved potency and selectivity for BLM compared to **ML216**. For example, the pyrazole-containing compound **33** exhibited an IC_{50} value of 1.1 μM against BLM and a greater than 6-fold selectivity over WRN (Figure S1). While compound **10** showed particularly good activity (IC_{50} = 0.10 μM) and a nearly 16-fold selectivity for BLM, it exhibited diminished solubility compared with **33**. All compounds showed only weak inhibition of RECQ1 at 50 μM. Based on its potency, selectivity, and *C*Log P, compound **33** was chosen to

further investigate the underlying mechanism of action and cellular activity for this class of compounds.

Table 5. RecQ helicase selectivity

	IC_{50} (μM)[a]			Selectivity Ratio
Compound	**BLM**	**WRN**	**RECQ1**	**BLM/WRN**
1	1.4	3.4	>50	2.4
7g	4.3	*ND*	*ND*	*ND*
10	0.10	1.6	*ND*	16
20	2.1	2.9	*ND*	1.4
23	0.11	1.1	>50	10
ML216	1.8	2.7	>50	1.5
28	0.30	2.2	>50	7.3
33	1.1	7.1	>50	6.5
36	1.8	*ND*	*ND*	*ND*

[a] Determined by gel-based DNA unwinding assay.
ND, not determined

Like most helicases, BLM exhibits intrinsic DNA-dependent ATPase activity, which provides energy for DNA strand separation. It can be envisioned that reduction in the accessibility of the ATP-binding site may lead to decreased ATP hydrolysis and an equivalent reduction of the DNA unwinding rate. To investigate this type of inhibition mechanism, we measured BLM-catalyzed ATP hydrolysis rates using a single-stranded oligonucleotide as the DNA effector in the presence of **33**. As shown in Figure 2A, kinetic analysis reveals that compound **33** does not affect the apparent K_m value of BLM ATPase activity. A Lineweaver-Burk double reciprocal plot of the curves derived from control and inhibitor-containing reactions gave regression lines that intersect at a point on the *x*-axis indicating that **33** is a non-ATP competitive BLM inhibitor (Figure 2B). These results also suggest that **33** acts by preventing BLM from binding to DNA, as demonstrated previously for **ML216**.[17] Indeed, dose-dependent inhibition of BLM binding to single-stranded DNA by **33** was confirmed using fluorescence polarization analysis (Figure S2).

Moreover, selective displacement of DNA binding to BLM over related DNA helicases was also observed for compound **33** (Figure S2).

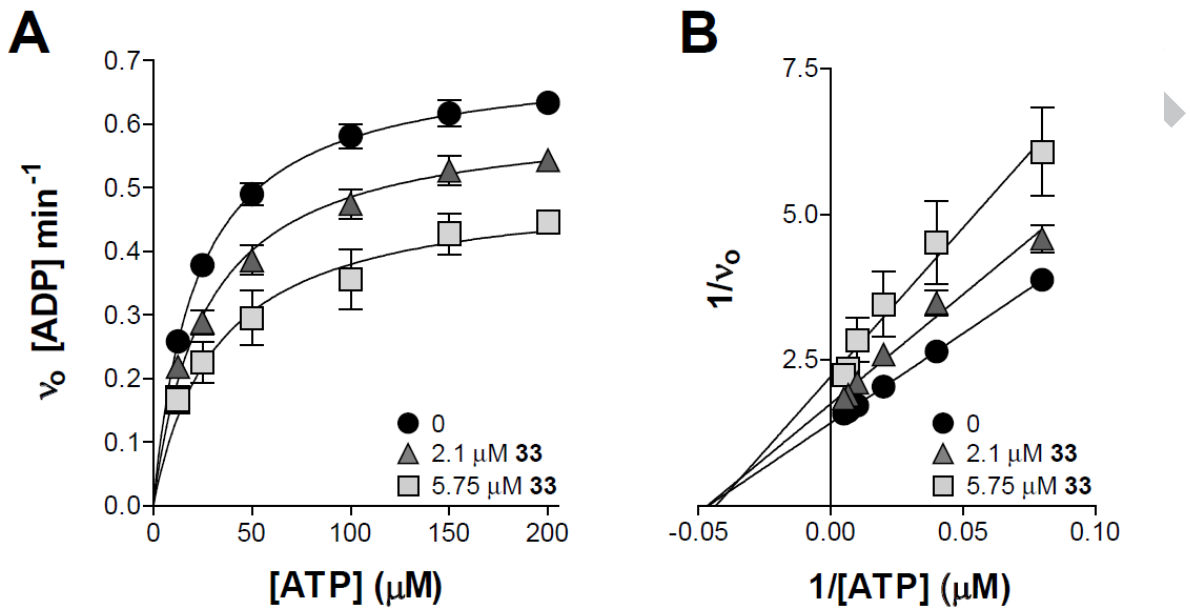


Figure 2. Inhibition mechanism of compound **33**. A) Michaelis-Menten kinetic analysis of BLM ATPase activity in the absence and presence of compound **33**. B) Lineweaver-Burke double reciprocal plot derived from the assay described in (A). Each data point represents the mean ± SD of quadruplicate determinations. The results are a representative of three independent experiments.

An elevated frequency of SCEs is a characteristic feature of BS cells. Thus, we next asked whether **33** could induce similar cytological effects in a BLM-specific manner. Using the isogenic pair of GM08505-derived BS cells,[21] we demonstrate a significant and dose-dependent induction of SCEs by **33** in cells expressing BLM (PSNF5), while no further increase in SCEs was observed in cells lacking BLM (PSNG13) (Figure 3). Given the intimate role of BLM in suppressing SCE formation, these results strongly suggest that the induction of SCEs by **33** is mediated through inhibition of BLM activity.

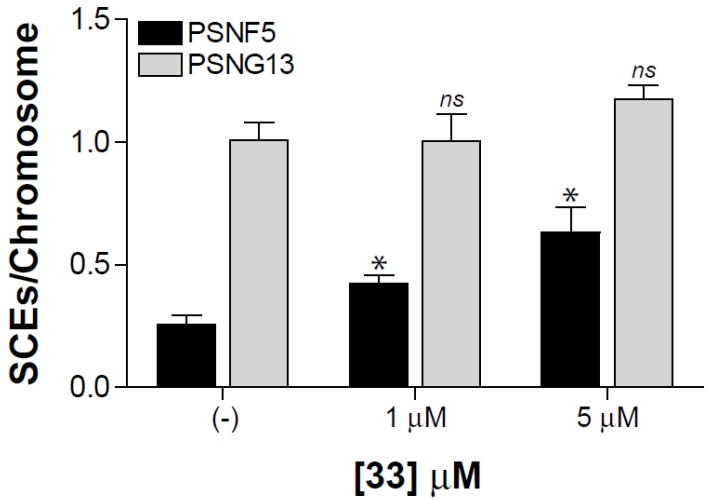


Figure 3. Effects of **33** on SCE frequency. BLM-complemented (PSNF5) and BLM-defective (PSNG13) cells were treated with **33**, as indicated below the bars, and the frequency of SCEs per chromosome was quantified. An asterisk indicates a p-value less than 0.01, while *ns* denotes no statistical significance ($p > 0.5$).

In order to gain a better understanding of the potential of these compounds to be used in studies beyond biochemical and cell-based assays we sought to determine representative *in vitro* ADME properties of the our top compounds (**ML216** and **33**). As shown in Table 6, these compounds exhibit generally favorable properties; namely microsomal stability, *C*Log P, and plasma stability. However, both compounds have low aqueous solubility (PBS buffer, pH 7.4) of 1 and 10 μM for **ML216** and **33**, respectively. Of note, the compounds show improved solubility in the assay buffer system (data not shown, *see* Supporting Information for buffer conditions), which suggests that the biochemical data for these analogs were not compromised by this liability. Typically, the improved solubility in the assay buffer is a result of having non-ionic detergent (Tween-20) present which aids in solubilizing the more lipophilic compounds. Moreover, both compounds have low Caco-2 permeability which may explain the higher concentrations of drug required to observe an effect in cell-based studies. The low solubility and permeability suggest that these compounds could possess poor absorption and oral bioavailability and further optimization of this chemotype may be required for use *in vivo*.

Table 6.[a] *In vitro* ADME properties for **ML216** and **33.**

Compound	*C*Log P	aq. Solubility (pH 7.4)	MLM Stability ($T_{1/2}$)	Plasma Stability (5 h)	Caco-2 Permeability	Efflux ratio
ML216	3.8	1 µM	>80 min	100%	1 X 10^{-6} cm/s	2
33	2	10 µM	>80 min	100%	ND	ND

[a]*C*LogP values were determined using ChemBioDraw™ 12.0; Solubility refers to the kinetic solubility as determined by Analiza Inc. (www.analiza.com) using chemiluminescent nitrogen detection (CLND) and in-house via UV-Vis detection methodology; MLM stability refers to mouse liver microsome stability in the presence of NADPH; Plasma stability was performed using mouse plasma and the % remaining after 5 hours was determined. The Caco-2 permeability value represents the P_{app} from A→B; Efflux ratio is B→A/A→B. Microsomal stability, Plasma stability and Caco-2 peremeability studies were conducted at Pharmaron Inc.

In summary, the medicinal chemistry optimziation efforts surrounding a 5-(pyridin-4-yl)-1,3,4-oxadiazol-2-amine based BLM helicase inhibitor is described. Top compounds possess low micromolar to sub-micromolar potency and good selectivity against other related DNA helicases. Moreover, the mode of inhibition was investigated and the activity in cell-based assays was demonstrated by an observation of an increase in SCEs, as anticipated. While some improvement in the aqueous solubility was achieved with compound **33**, compared to the previously described **ML216**, this particular ADME attribute remains a liability. We hope that the compounds described herein provide a means to interrogate BLM helicase biology through pharmacological inhibition and offer them freely to the research community.

Acknowledgements

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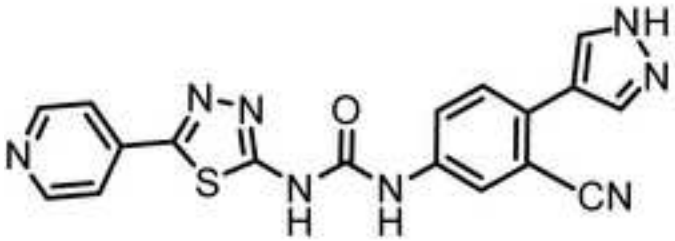
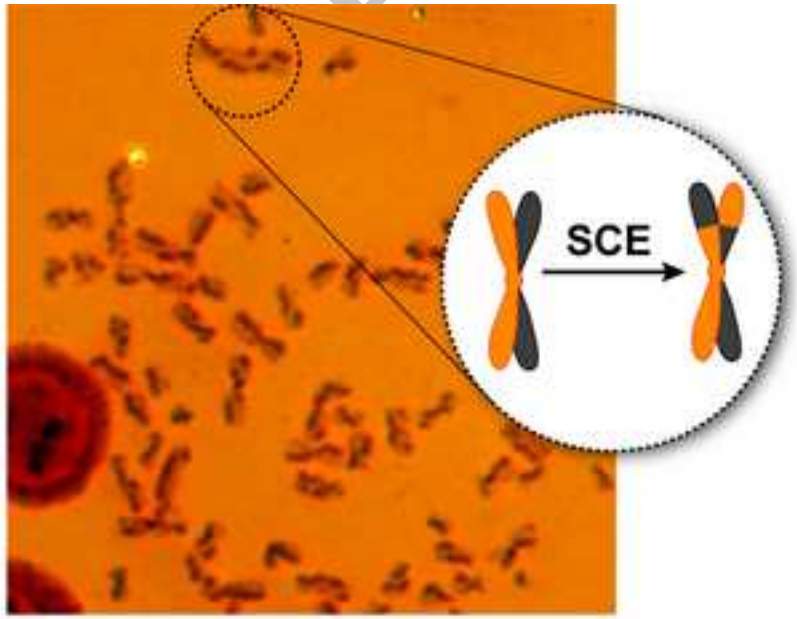


33: BLM IC_{50} = 1.1 μM

BLM Helicase Inhibition

↑SCE



Sister Chromatid Exchange (SCE)