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2-Anilino-4-aryl-1,3-thiazole inhibitors of valosin-containing protein (VCP or p97)

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ABSTRACT

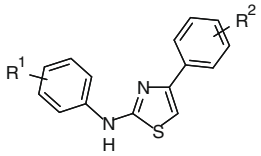
Valosin-containing protein (VCP; also known as p97) is a member of the AAA ATPase family with a central role in the ubiquitin-degradation of misfolded proteins. VCP also exhibits antiapoptotic function and metastasis via activation of nuclear factor kappa-B signaling pathway. We have discovered that 2-anilino-4-aryl-1,3-thiazoles are potent drug-like inhibitors of this enzyme. The identified compounds show low nanomolar VCP potency, demonstrate SAR trends, and show activity in a mechanism based cellular assay. This series of compounds represents the first steps towards a novel, small molecule VCP inhibitor as a cancer therapeutic.

Valosin-containing protein (VCP; also known as p97) is a member of the AAA ATPase family that plays a role in several biological processes. These include ERAD (endoplasmic reticulum associated degradation), nuclear membrane fusion after completion of mitosis, golgi reassembly, apoptosis repression, and activation of transcription factors.[1] VCP also plays a central role in removing ubiquitin-tagged, misfolded proteins from the endoplasmic reticulum and escorting them to the 26S proteasome for degradation.[2]

VCP protein is over expressed in gastric, colon, pancreatic, and hepatocellular cancers.[1] Overabundance of VCP has been shown to target IκB for degradation, thereby activating the NFκB pathway. Activation of the NFκB pathway is commonly observed during tumorigenesis.[3] Disruption of VCP function, via RNA interference or over expression of ATPase deficient protein in tumor cell lines, has been shown to cause cell death.[4,5] Thus, a VCP inhibitor has the potential as a novel small molecule cancer therapeutic.

A high throughput screening (HTS) assay for VCP inhibitors was therefore developed to identify an entry point for lead identification/optimization. Inhibition of ATPase activity was determined by a glucokinase coupled enzyme assay.[6–8] Conversion of ATP to ADP was detected by resorufin signal and VCP inhibition confirmed by an ATPase assay.

Here we report the discovery and structure–activity relationships (SAR) of thiazole-based VCP inhibitor **1** found via HTS. To our knowledge, this class of compounds represents the first disclosure of small, drug-like VCP inhibitors with potential utility as a cancer therapeutic.



1

Initial efforts to explore the 2-anilino-4-aryl-1,3-thiazole VCP inhibitor scaffold began with the Hantzsch thiazole synthesis outlined in Scheme 1.[9,10] Various commercial arylthioureas **2** and readily or commercially available α-bromo ketones **3** were cyclized in refluxing EtOH to afford the desired 2-anilino-4-aryl-thiazole **1**.

Based on our work with another scaffold (data not shown), we first investigated R^1 substitution while holding R^2 constant (R^2 = *p*-Cl). VCP inhibition data for this initial set of 2-anilino-4-aryl-1,3-thiazoles (**4**–**18**) is shown in Table 1. Compounds **4**–**18** demonstrate that the R^1 aniline substitution plays a key role in VCP activity. When R^1 = H or a *meta* substituent, minimal VCP inhibition is seen (R^1 = H **4**: 14% inhibition; R^1 = 3-OH, 3-CN, or 3-CF_3 **5**–**7**: 26% inhibition, 26% inhibition, and IC_{50} = 8.3 μM, respectively). At-

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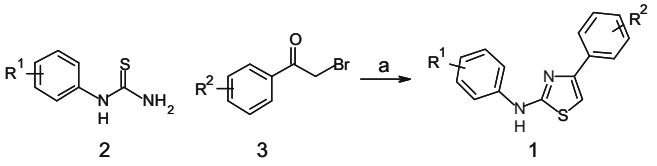
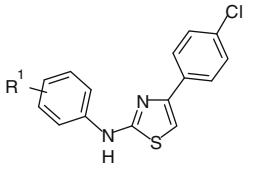


Scheme 1. Reagents and conditions: (a) EtOH, reflux.

Table 1
VCP inhibition for various R^1 substituents of 2-anilino-4-(*para*-chloro)phenyl-1,3-thiazoles



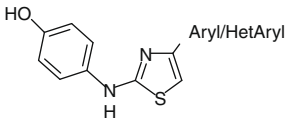
Compd	R^1	VCP IC_{50}[a] (μM)	VCP% inhib[b]
4	H		14
5	3-OH		26
6	3-CN		25
7	3-CF_3	8.3	
8	3-CO_2H		35
9	3-NHAc		25
10	4-CN		10
11	4-NO_2		15
12	4-OH	0.07	
13	4-NH_2	0.08	
14	4-OMe		7
15	4-NHAc	>10	
16	4-CO_2H		40
17	4-$CONH_2$	>20	
18	4-CONMe		25

[a] Values are means of two experiments, standard deviations are ±10%.
[b] Values are VCP percent inhibition at 10 μM inhibitor concentration.

tempts to display hydrogen bond donors/acceptors in the R^1 *meta* position yielded minimally active VCP analogs (R^1 = 3-CO_2H **8**: 35% inhibition; R^1 = 3-NHAc **9**: 25% inhibition). When R^1 was a *para* substituent a wide range of VCP activity was observed. Electron withdrawing substituents show minimal activity (R^1 = 4-CN **10**: 10% inhibition; R^1 = 4-NO_2 **11**: 15% inhibition). Rewardingly, hydrogen bond donating substituents showed much improved potency (R^1 = 4-OH **12**: IC_{50} = 0.07 μM; R^1 = NH_2 **13**: IC_{50} = 0.08 μM). Masking these hydrogen bonding substituents led to greatly reduced potency (R^1 = 4-OMe **14**: 26% inhibition; R^1 = NHAc **15**: IC_{50} > 10 μM). Other attempts at adding hydrogen bond donors/acceptors in the R^1 *para* position yielded minimally active VCP analogs (R^1 = 4-CO_2H **16**: 40% inhibition; R^1 = 4-$CONH_2$ **17**: IC_{50} > 20 μM; R^1 = 4-CONHMe **18**: 25% inhibition). Other attempts to replace the *p*-OH or *p*-NH_2 with fused heterocycles such as indoles, indazoles, benzimidazoles, benzoxazoles, and benzothiazoles (not shown) led to minimally active VCP analogs.

To expand upon the identification of **12** (VCP IC_{50} = 0.07 μM) a series of 2-(*para*-hydroxy)-anilino-4-aryl thiazoles was synthesized (Table 2). While much SAR was developed around the 4-chloro phenyl, a more potent replacement was not found. The unsubstituted phenyl **19**, and isosteric 2-thiophene analog **20**, show nanomolar VCP activity (IC_{50} = 0.19 μM and 0.11 μM, respectively). The three regioisomeric 2-, 3-, and 4-pyridines showed reduced activity (**21**–**23**: IC_{50} = 0.23 μM, 0.30 μM, and 0.29 μM, respectively). The larger 2-naphthyl substituent resulted in even greater loss of activity (**24**: IC_{50} = 1.3 μM). Various substituted phenyl analogs **25**–**42** were then investigated. Both the fluoro and

Table 2
VCP inhibition for various 2-(*para*-hydroxy)anilino 4-aryl/heteroaryl-1,3-thiazoles



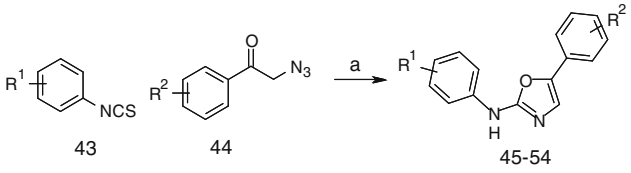
Compd	Aryl/hetaryl	VCP IC_{50}[a] (μM)	VCP% inhib[b]
19	Phenyl	0.19	
20	2-Thiophene	0.11	
21	2-Pyridyl	0.23	
22	3-Pyridyl	0.30	
23	4-Pyridyl	0.29	
24	2-Naphthyl	1.30	
25	(2-F) Phenyl	0.36	
26	(2-OMe) Phenyl	0.67	
27	(2-CF_3) Phenyl	2.7	
28	(2-CO_2H) Phenyl		44
29	(3-Cl) Phenyl		53
30	(3-CN) Phenyl		26
31	(3-OMe) Phenyl		27
33	(3-NO_2) Phenyl	0.76	
34	(3-OH) Phenyl	0.12	
35	(4-CN) Phenyl	0.18	
36	(4-CF_3) Phenyl		9
37	(4-Me) Phenyl		55
38	(4-OMe) Phenyl	2	
41	(4-Morpholine) Phenyl		13
42	(3,4-di-Cl) Phenyl	0.43	

[a] Values are means of two experiments, standard deviations are ±10%.
[b] Values are VCP percent inhibition at 10 μM inhibitor concentration.

methoxy *ortho*-substituted phenyl analogs **25** (IC_{50} = 0.36 μM) and **26** (IC_{50} = 0.67 μM) showed sub-micromolar VCP activity. Conversely, the electron withdrawing trifluoromethyl and carboxylic acid phenyl analogs **27** and **28** showed reduced VCP activity (IC_{50} = 2.7 μM and 44% inhibition, respectively). Various *meta*-substituents also provided a range of VCP activities. The chloro, cyano, and methoxy *meta*-substituted phenyl analogs **29**–**31** showed reduced VCP activity (53%, 26%, and 27% inhibition, respectively). However, the nitro (**32**: IC_{50} = 0.76 μM) and hydroxyl (**33**: IC_{50} = 0.12 μM) *meta*-substituted phenyl analogs showed nanomolar VCP activity. *Para*-substituted aryl analogs **35**–**41** also provided a range of VCP activities. The cyano and methoxy *para*-substituted phenyl analogs, **35** and **38**, provided VCP active compounds (IC_{50} = 0.18 and 2.0 μM, respectively). Conversely, other *para*-substituted phenyl substituents led to minimally active VCP analogs **36**, **37**, and **41** (9%, 55%, and 31% inhibition, respectively). The *meta*, *para*-disubstituted phenyl analog **42** (3,4-dichloro) afforded a compound with sub-micromolar potency (IC_{50} = 0.43 μM).

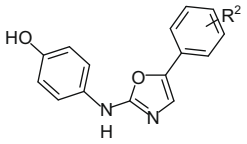
We then turned our efforts toward exploring a replacement of the central thiazole core with an oxazole. This work began with the synthetic route outlined in Scheme 2.[9,11] Heating commercially available *p*-OH phenyl isothioisocyanate **43** with various α-azidoacetophenones **44** (prepared from commercially available acetophenones) in the presence of resin-bound triphenyl phosphine afforded the desired 2-anilino-5-aryl-oxazoles **45**–**54**.

VCP inhibition data for this set of oxazoles **45**–**54** is shown in Table 3. In general, the oxazoles investigated showed nanomolar



Scheme 2. Reagents and conditions: (a) PPh_3 resin, dioxane, reflux.

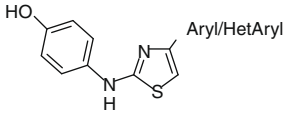
Table 3
VCP inhibition for various 4-aryl-2-(*para*-hydroxy) anilino-4-aryl-1,3-oxazoles



Compd	R^2	VCP IC_{50}[a] (μM)	VCP% inhib[b]
45	H	0.091	
46	2-OMe	0.12	
47	2-F		7
48	3-Cl	0.14	
49	3-OMe	0.17	
50	3-CO_2H	0.19	
51	4-Cl	0.33	
52	4-Me		16
53	4-Morpholine	0.13	
54	4-NEt_2	0.23	

[a] Values are means of two experiments, standard deviations are ±10%.

Table 4
VCP cellular inhibition for various 2-anilino-4-aryl/heteroaryl-1,3-thiazole inhibitors of VCP



Compd	Aryl/hetaryl	VCP IC_{50}[a] (μM)	Luciferase stabilization EC_{50}[a,b] (μM)
12	(4-Cl) Phenyl	0.07	<1.20
19	Phenyl	0.19	0.52
20	2-Thiophene	0.11	0.09
21	2-Pyridyl	0.23	<1.20
34	(3-OH) Phenyl	0.12	0.20

[a] Values are means of two experiments, standard deviations are ±10%.
[b] EC_{50} = concentration that results in half-maximal stabilization of the Luciferase reporter construct in HeLa cells. Values are means of two experiments, standard deviations are ±10%. More accurate values for **12** and **21** could not be calculated due to cell killing.

VCP inhibitory activity, but the SAR showed differing trends than the related thiazoles. The unsubstituted phenyl derivative **45** (IC_{50} = 0.09 μM) demonstrated low nanomolar VCP potency. While the *ortho*-fluoro analog (**47**: 7% inhibition) showed minimal activity, the electron donating *ortho*-methoxy phenyl analog **46** (IC_{50} = 0.12 μM) showed similar low nanomolar activity. All three *meta*-substituted analogs (**48**–**50**: chloro, methoxy, and carboxcylic acid, respectively) showed potent nanomolar inhibitory activity. The *para*-chloro analog, which was the most potent thiazole, possessed reduced VCP activity (**51**: IC_{50} = 0.33 μM) and the *para*-methyl phenyl analog **52** (7% inhibition) demonstrated only minimal activity. Both *para*-substituted amino analogs (**53**–**54**: morpholine and diethyl amine, respectively) also showed nanomolar VCP activity.

To further profile these VCP inhibitors several of the more potent compounds were evaluated in a mechanism based cellular assay shown in Table 4. This assay utilized a ubiquitin-tagged Luciferase reporter (Ub-G76 V-Luciferase) as VCP has been shown to play a role in targeting ubiquitinated fusion constructs for degradation.[2,12] Several of the potent thiazole-based VCP inhibitors cause stabilization of the reporter construct in HeLa cells after a 24 h treatment with low micromolar (**12** and **21**) to nanomolar (**19**, **20**, and **34**) cellular activity.[13]

In conclusion, we have presented a series of 2-anilino-4-aryl-1,3-thiazole VCP inhibitors. These thiazoles and the related oxazoles have been synthesized in a parallel synthesis approach to investigate a HTS-derived class of inhibitors. The compounds presented herein are small, drug-like lead molecules with tractable SAR and low nanomolar potency against an emerging oncology target, VCP. Several potent VCP inhibitors also show sub-micromolar activity in a mechanism based cellular assay. The continued development of novel, small molecule VCP inhibitors towards a cancer therapeutic will be reported in due course.

Acknowledgments

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