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Synthesis and structure–activity relationship of (1-halo-2-naphthyl) carbamate-based inhibitors of KIAA1363 (NCEH1/AADACL1)

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ABSTRACT

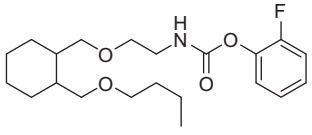
KIAA1363 is a serine hydrolase whose activity has been shown to be positively associated with tumor cell invasiveness. Thus, inhibitors of KIAA1363 represent a novel targeted therapy approach towards cancer. **AX11890** ((1-bromo-2-naphthyl) *N*,*N*-dimethylcarbamate) was identified as a KIAA1363 inhibitor with an IC_{50} value of 1.2 μM and was shown using ESI-MS to carbamylate the catalytic residue Ser^{191}. SAR studies explored both substitution of the 1-bromo group and derivatization of the 6-position. Activity-based protein profiling demonstrated **AX13057** inhibited tumor-localized KIAA1363 in SK-OV-3 xenograft-bearing mice.

KIAA1363 (gene name NCEH1; the protein is also known as neutral cholesterol ester hydrolase 1 and arylacetamide deacetylase-like 1 [AADACL1]) is a serine hydrolase whose activity has been shown to be positively associated with tumor cell invasiveness.[1] KIAA1363 activity has also been found to be increased in clinical breast tumor biopsies relative to normal breast tissue, and furthermore to be higher in prognostically worse ER/PR double negative tumors than in double positive tumors.[2] KIAA1363 mediates the hydrolysis of 2-acetyl monoalkylglycerol, a metabolic intermediate in an ether lipid pathway producing the biologically active lipid, alkyl-LPA.[3] Inhibition of KIAA1363 by RNA knockdown or by use of inhibitors has been shown to reduce alkyl-LPA and to disrupt cellular processes such as migration[3] or tumor growth in vivo.[4] Thus, inhibitors of KIAA1363 activity represent a novel targeted therapy approach towards cancer. Various KIAA1363 inhibitors have been reported in the literature,[5–7] including the carbamate **AS115**[3] (Fig. 1).

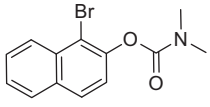
As part of an effort to identify novel KIAA1363 inhibitors we conducted a high throughput screen of an internal compound library which led to the identification of the carbamate **AX11890** (Fig. 1) as a 1.2 μM inhibitor of KIAA1363.[8] As part of a hit-to-lead optimization effort, we set out to better understand the structure–activity relationship (SAR) of the hit and to improve its potency by modifying both the 1-position and 6-position. The result of this effort was the identification of **AX13057** (Table 3), a tumor-penetrant KIAA1363 inhibitor.

An initial set of compounds (Table 1) was examined to explore the SAR of the HTS hit **AX11890**. When the 1-bromo group was removed (**2**)[9] or moved to the 3-position (**3**) potency decreased. Moving the bromine to the 6-position (**4**)[9] resulted in a compound with similar KIAA1363 inhibitory activity to **AX11890**. These results indicated that substitution at the 1 and 6 positions were important drivers for potency. Truncated versions of the **AX11890** in which the unsubstituted phenyl ring was removed (**5**)[10] or replaced by two methyl groups (**6**) showed a dramatic reduction in potency. This latter data was taken to demonstrate that the bicyclic naphthalene core was an important pharmacophore element.

Replacements for the 1-bromo group of **AX11890** were examined to find analogs with improved potency (Table 2). The synthe-



AS115 (*cis*)



AX11890

Figure 1. The structure of **AS115** and the KIAA1363 HTS hit **AX11890**.

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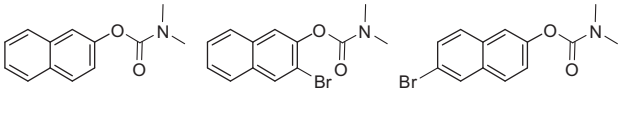
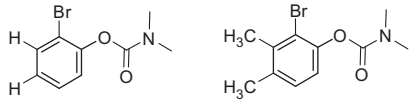
Table 1
KIAA1363 IC_{50} values (20 min pre-incubation) for the HTS hit **AX11890** and compounds **2**–**6**



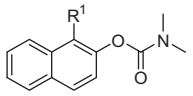
2 **3** **4**



5 **6**

Compound	KIAA1363 IC_{50} (μM)
AX11890	1.2
2	>100
3	55
4	0.92
5	>100
6	24

Table 2
KIAA1363 IC_{50} values (20 min pre-incubation) for 1-position variants of the HTS hit **AX11890**

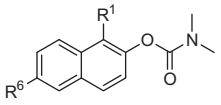


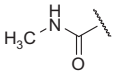
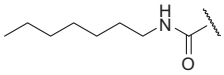
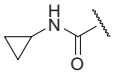
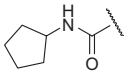
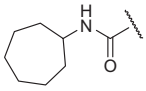
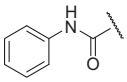
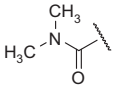
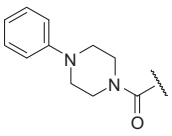
Compound	R^1	KIAA1363 IC_{50} (μM)
AX11890	–Br	1.2
7	$-CH_3$	23
8	$-CH_2CH_3$	6.8
9	$-CH_2CH_2CH_3$	3.4
10	$-CH_2CH_2CH_2CH_3$	7.5
11	$-CH(CH_3)_2$	21
12	$-NH_2$	69
13	$-COCH_3$	11
14	$-CO_2Me$	3.9
15	CN	>100
16	–Cl	1.1
17	$-NO_2$	0.89
18	–I	0.33

sis of such analogs began with 1-substituted-2-naphthol derivatives which were synthesized according to literature procedure[11] or purchased commercially. Subsequent carbamylation with dimethylcarbamyl chloride (Me_2NCOCl) in Cs_2CO_3/acetone (see an exemplary synthesis in Scheme 1) yielded the compounds in Table 2. Replacement of the 1-bromo group with linear or branched alkyl groups (**7**–**11**), a primary amine (**12**), or various electron withdrawing groups (**13**–**15**) offered only a decrease in potency relative to the HTS hit. By contrast, the 1-chloro derivative **16** was equipotent with **AX11890**. Only the nitro (**17**) and iodo (**18**) groups afforded an increase in potency with the latter being the most potent inhibitor in this series having a KIAA1363 IC_{50} value of 330 nM.

Derivatization of **AX11890** at the 6-position was also explored (Table 3). When several 6-carboxamide derivatives were synthesized (Scheme 1) and examined in the KIAA1363 IC_{50} assay, SAR data emerged indicating more hydrophobic or larger amides were often preferred. For example, extending the *N*-methyl amide **19** to *N*-heptyl (**20**) resulted in a 17-fold increase in potency. Similarly,

Table 3
KIAA1363 IC_{50} values (20 min pre-incubation) for 6-position derivatives of the HTS hit **AX11890**

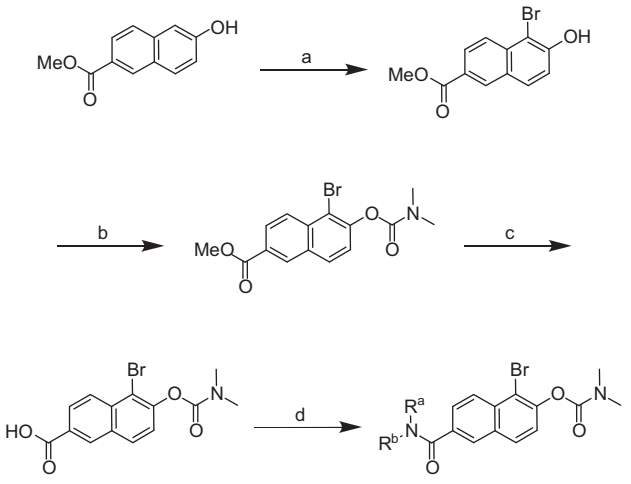


Compound	R^6	R^1	KIAA1363 IC_{50} (nM)
19		–Br	29,000
20		–Br	1700
21		–Br	17,000
22		–Br	1500
23		–Br	920
24		–Br	1300
25		–Br	11,000
26		–Br	310
27	–CN	–Br	150
AX13057	$-CH_2CH_2CH_3$	–Br	130
28	–Br	–Br	50
29	-CN	–Cl	150
30	$-CH_2CH_2CH_3$	–Cl	150
31	–Br	–Cl	80

expanding the ring size of the *N*-cyclopropyl amide **21** resulted in a concomitant increase in KIAA1363 inhibitory activity (compare **21** through **24**). Finally, annulation of the *N*,*N*-dimethylamide **25** gave the most potent inhibitor (**26**) among the 6-carboxamides explored with a KIAA1363 IC_{50} of 310 nM.

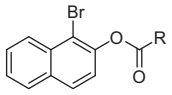
When cyano (**27**), *n*-propyl (**AX13057**), and bromo (**28**) groups were incorporated into the 6-position of **AX11890**, the most dramatic increases in potency were observed (7- to 21-fold). Indeed, the most potent inhibitor identified in this study, the 1,6-dibromo derivative **28** (KIAA1363 IC_{50} = 50 nM), was a fusion between the ~1 μM KIAA1363 inhibitors **AX11890** and **4**. Three 1-chloro analogs (**29**–**31**) of the most potent 1, 6-disubstituted inhibitors described above were also investigated and found to be of similar potency as their 1-bromo congeners. Thus, the 6-position was further established as a driver for potency.

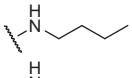
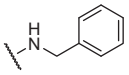
ESI-MS was used to confirm carbamylation as a mechanism of action for **AX11890**. Incubation of a KIAA1363-expressing cell membrane fraction with 100 μM **AX11890** was followed by gel filtration to remove any trace of small molecule inhibitor detectable by MS. After tryptic digestion and MS^2 sequencing, both the un-modified active site peptide and the same peptide *N*,*N*-dimethyl carbamylated at the active site serine 191 was observed (see Supplementary data).[12] Because covalent inhibitors are often associated with time-dependent inhibition, we examined



Scheme 1. Synthesis of 6-carboxamide derivatives of **AX11890**. Reagents and conditions: (a) NBS (1.0 equiv), DMF, rt, 1 day, 89%; (b) Me_2NCOCl (1.15 equiv), Cs_2CO_3 (1 equiv), acetone, rt, 1 day, 89%; (c) LiOH (10 equiv), THF/H_2O (1:1), rt, 1 day, 90%; (d) CDI (1 equiv), CH_3CN then amine (2 equiv), rt, 3 days.

Table 4
KIAA1363 IC_{50} values measured within the same experiment (**A**: 20 min pre-incubation time and **B**: 4 h pre-incubation time) for the HTS hit **AX11890**, carbamate analogs of **AX11890**, and **AS115**. The last column is the ratio between the two pre-incubation times for each compound and is shown as a comparative measure of time-dependent inhibition



ID	R	KIAA1363 IC_{50} (μM)—A	KIAA1363 IC_{50} (μM)—B	A/B
1		1.8	1.7	1.1
32		2.0	0.33	6.1
33		37	2.2	17
34		28	1.5	30
35		27	0.71	37
36		42	1.1	38
AS115		5.4	0.10	54

the potency of **AX11890** using both a 20 min and 4 h pre-incubation time. To our surprise, the KIAA1363 inhibitory activity of **AX11890** showed no significant increase in potency with a longer pre-incubation time (Table 4). This behavior was in contrast to carbamate analogs of **AX11890** (**32–36**)[13], including the closely related azeditine carbamate **32**, all of which exhibited time-dependent inhibition. As a control, **AS115** was examined and demonstrated the largest degree of time-dependent inhibition with a KIAA1363 IC_{50} value of 100 nM at the 4-h pre-incubation time. This value was close to the reported **AS115** KIAA1363 IC_{50} value of 150 nM determined using a serine hydrolase activity-based probe assay.[3]

Decarbamylation of KIAA1363 could be monitored by denaturing the covalently modified enzyme at different time points and

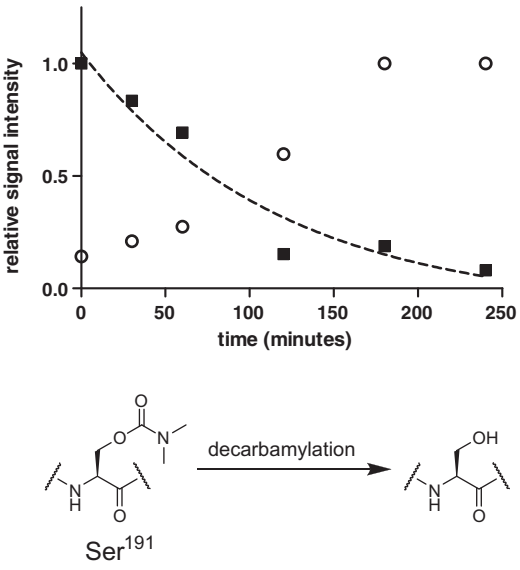


Figure 2. Plot of the diminishing signal intensity of the *N*,*N*-dimethyl carbamylated KIAA1363 active site tryptic peptide (■) and the corresponding rise of the same unmodified peptide (○) as a function of time. For the former, the best fit to a single exponential decay is shown to yield a $t_{1/2}$ value of 66 min. Below the plot is a scheme showing the decarbamylation of the active site serine (i.e. conversion of ■ to ○).[10]

Table 5
KIAA1363 inhibition measured using activity-based protein profiling with **AX8891** after dosing compounds **27**, **28**, and **AX13057** (po or ip, 100 mg/kg) to SK-OV-3 xenograft bearing female SCID mice

Compound	Route of administration	Tissue	% Inhibition KIAA1363
27	po	Tumor	14
28	po	Tumor	18
AX13057	po	Tumor	22
AX13057	ip	Tumor	64
AX13057	ip	Heart	66

plotting the signal strength of the resultant carbamylated active site peptide (see Fig. 2). When fit to a single-exponential, a $t_{1/2}$ value of 66 min was measured for the *N*,*N*-dimethylcarbamylated species, a relatively short value compared to the length of the time-dependent assay. When this same set of experiments was conducted with **AS115**, the expected active site carbamate was observed and found not to decarbamylate after 4 h (data not shown). On the continuum of covalent inhibitors, **AX11890** can be described as a covalent yet reversible inhibitor (i.e. pseudo-irreversible and substrate-like) whereas **AS115** more closely approximates an irreversible inhibitor. In total the data demonstrates the structure of carbamate can play a role in modulating the rate of enzymatic decarbamylation, an observation noted before with inhibitors of cholinesterase.[14]

Finally, the ability of selected KIAA1363 inhibitors (**27**, **28**, and **AX13057**) to inhibit KIAA1363 in SCID mice bearing SK-OV-3 human ovarian cancer xenografts was examined (see Table 5)[15] Post oral administration (100 mg/kg), all 3 compounds inhibited the labeling of intratumoral human KIAA1363 by a fluorophosphonate serine hydrolase activity-based probe. Because **AX13057** gave the highest level of inhibition (22%), it was re-examined via an intraperitoneal route of dosing to maximize exposure. Inhibition values of 64% and 66% were measured for intratumoral human KIAA1363 and mouse heart KIAA1363, respectively. Inhibition of murine heart KIAA1363 could also be examined as a pharmacodynamic assay for in vivo inhibitor efficacy in non-tumor bearing mice.

In summary, the SAR studies described herein were used to define the important pharmacophore elements of (1-halo-2-naphthyl) carbamates as a novel class of KIAA1363 inhibitors. Activity-based protein profiling demonstrated that **AX13057** inhibited intratumoral KIAA1363 in SK-OV-3 xenograft-bearing mice. All data taken together, (1-halo-2-naphthyl) carbamates offer promise as a starting point in the development of KIAA1363 inhibitors. Lead optimization based on this motif will be reported in due course.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.102.

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7. Chang, J. W.; Moellering, R. E.; Cravatt, B. F. *Angew. Chem., Int. Ed.* **2012**, *51*, 966.
8. *KIAA1363 IC_{50} assay*: The human KIAA1363 cDNA clone was a kind gift from Dr. Benjamin Cravatt (The Scripps Research Institute). KIAA1363 was expressed in human 293T/17 cells (ATCC) and the membrane fraction was isolated by ultracentrifugation at 100,000×*g* for 1 h at 4 °C followed by resuspension using sonication in 50 mM Tris pH 7.4. For the assay, membranes were diluted to a final concentration of 0.1 mg/ml in 50 mM Tris pH 7.4 + 5 mM CHAPS. Membranes were pre-incubated with compound at room temperature prior to adding 200 μM 2-thioacetyl MAGE substrate (Cayman Chemical). For experiments in which a 20 min pre-incubation was used, 200 μM DTNB was present throughout the pre-incubation. For experiments in which a 4 h pre-incubation was used, 200 μM DTNB was added at the time of substrate addition. The final concentration of DMSO was 4% in a total reaction volume of 50 μL. Reactions were allowed to proceed for 1 h at 37 °C, and absorbance at 405 nm was measured using a Wallac Victor plate reader. All compounds were tested in duplicate. IC_{50} values were determined using GraphPad Prism.
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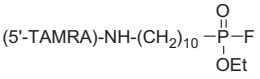
12. KIAA1363/293T/17 membrane in 50 mM Tris, pH 7.4 (0.15 mg in 50 μL) was incubated with 100 μM of KIAA1363 inhibitor for 1 h at room temperature. The resulting membranes (75 μL) were eluted through a Micro Bio-Gel P-30 column to remove excess inhibitor. The eluate was denatured with urea (6 M), reduced with DTT (1 M), and alkylated with iodoacetamide (1 M). Theses samples were eluted through a Micro Bio-Gel P-30 column and the resulting eluate digested with trypsin overnight. The digested samples were analyzed with capillary HPLC (Agilent Technologies) and LTQ-linear Ion trap mass spectrometer with nano-ESI source (Thermo Finnigan). Ten microlitres of each sample was desalted and concentrated on a peptide capTrap (Michrom Bioresources). Peptides then were separated on a 0.18 × 100 mm, C18, 5 μm reversed phase column with a gradient of 5% ACN, 0.1% formic acid to 50% ACN, 0.1% formic acid in 2 h with column flow of 1.8 μL/min and injected to the LTQ mass spectrometer. Data was acquired in target MS/MS mode on the active site peptide (ICISGDS191AGGNLAAALGQQFTQDASLK) and its carbamylated form (ICISGD[S191-H+CONMe2]AGGNLAAALGQQFTQDASLK) with the following settings: spray voltage: 1.8 kV, capillary temperature: 200 °C, capillary voltage: 46 V, tube lens voltage: 120 v, relative collision energy: 35%. Peptide fragments were identified with TurboSEQUEST (Thermo Finnigan). Post incubation with AX11890 the signal intensity ratio of carbamylated active site peptide to un-modified active site peptide was 13.
13. These compounds were produced by the reaction of 1-bromo-2-naphthol with 4-nitrophenyl chloroformate (1 equiv) in DIPEA/DCM at 0 °C followed by treatment with the appropriate amine (1 equiv) at room temperature overnight. Secondary carbamates were found to exhibit chemical instability. For example, the prolonged storage of such compounds in DMSO at −78 °C resulted in decomposition. No such chemical instability was seen with tertiary carbamates.
14. (a) Rampa, A.; Bisi, A.; Valenti, P.; Recanatini, M.; Cavalli, A.; Andrisano, V.; Cavrini, V.; Fin, L.; Buriani, A.; Giusti, P. *J. Med. Chem.* **1998**, *41*, 3976; (b) Rampa, A.; Piazzi, L.; Belluti, F.; Gobbi, S.; Bisi, A.; Bartolini, M.; Andrisano, V.; Cavrini, V.; Cavalli, A.; Recanatini, M.; Valenti, P. *J. Med. Chem.* **2001**, *44*, 3810; (c) Bartolini, M.; Cavrini, V.; Andrisano, V. *J. Chromatogr., A* **2007**, *1144*, 102.
15. In vivo experiments were carried out at Explora Biolabs (San Diego, CA) in accordance with the guidelines of the Institutional Animal Care and Use Committee. Female SCID mice bearing SK-OV-3 ovarian cancer xenografts of an average volume [½ (length × width^2)] of 115–700 mm^3 were used for PD studies. Compounds were resuspended in PEG400 and 100 μL was dosed at 100 mg/kg according to average body weight. At 2 h after dosing, mice were sacrificed and tumors and/or hearts were removed, weighed, and snap frozen in liquid nitrogen. Tissues were individually homogenized in 50 mM Tris pH 7.4, and centrifuged at 1000×*g* for 10 min at 4 °C. The supernatant was then collected and briefly sonicated on ice. Protein concentrations were determined using the BioRad Dc protein kit and adjusted typically to 5 mg/mL. An aliquot of each lysate was labeled with the activity-based probe AX8891 at 1 μM for 90 s. The reaction was terminated by the addition of an equal volume of 2×SDS–PAGE loading buffer and heating at 95 °C for 5 min. To determine total and background (no KIAA1363 activity) labeling, equal volumes of protein-adjusted lysates from control group animals were pooled and preincubated with DMSO or 10 μM AX13057 inhibitor, respectively, prior to activity-based probe labeling. Thirty-five μg of each sample was loaded onto 12.5% SDS–PAGE gels along with at least duplicate loadings of the total and background controls. Resolved gels were directly scanned for fluorescence using a 605 nm bandpass filter for detection on the Hitachi FMBIO IIe (MiraiBio). Fluorescence was measured using ImageAnalysis software for KIAA1363 activity and expressed as fluorescence volume units.



AX8891