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2-Aminothiazole Derivative as a New Class of TrkA Kinase Inhibitor†

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Cancer (also called a malignant neoplasm) is a class of diseases in which a group of cells show uncontrolled cell growth, invasion and occasionally metastasis, and is a major public health problem worldwide. However, drug discovery and the development of cancer therapeutics take many years before a patient can benefit from a new drug. This time period is too long and needs to be shortened so patients can benefit quickly from new technologies and product development ideas.

Combinatorial chemistry is a widely used technique for accelerating drug discovery and development, in which tools and technologies from biology and chemistry are used in a parallel manner. It was reported that many 2-aminothiazoles exhibit antitumor activity through the inhibition of kinases,[1-3] Therefore, in the course of searching for anticancer agents, 2-aminothiazole derivatives were recently prepared by varying the 2-amino position and 5-substituted group in a high-speed parallel format according to the literature procedure, as shown in Scheme 1.[4] Among synthesized, compound **H154** exhibited significant cell growth inhibition against Hep3B cells with an IC_{50} value of 0.040 μM by the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) assay. However, the specific cellular mechanism of action of this compound is unclear.[5]

To investigate the mechanism of action and the kinase inhibitory profile of **H154**, the compound was tested on a panel of 109 cancer related kinases using the kinase profiling service at Milipore,[6] because 2-aminothiazole derivatives has inhibitory activity against the kinases by competing for the ATP binding site.[1-3] When the inhibitory activity of this compound was investigated across the different protein kinase at 10 μM, among the 109 different protein kinases, **H154** inhibited the activity of TrkA (Troponyosin-receptor kinase A) significantly. Accordingly, compound **H154** was further tested for its ability to activate the 6His-tagged human Trk cytoplasmic domain in a 10-dose IC_{50} mode with three-fold serial dilutions starting at 1 μM.[7] The results showed that **H154** has significant potency with an IC_{50} of 13 nM, as shown in Figure 1.

Among the Trk kinases inhibitors, staurosporine and its subclass, such as K-252a and CEP-701, have been discovered to be ATP competitive kinase inhibitors with a nonomolar

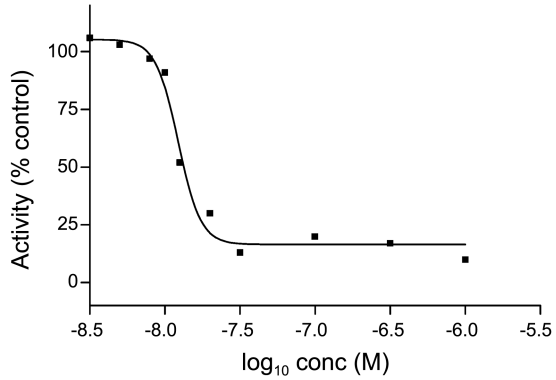
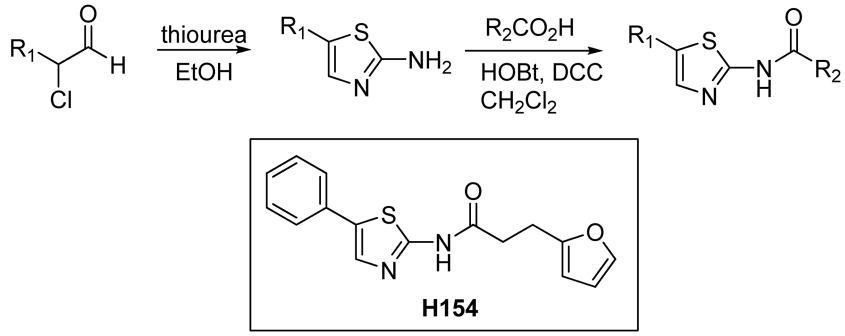


Figure 1. Inhibition of the TrkA kinase activity by compound **H154**. The enzymatic activity of *N*-terminal 6His-tagged recombinant human TrkA (residues 440-end, 41kDa) was measured in the presence of an increasing concentration of **H154**. The IC_{50} value of **H154** (average of 3 determinations) was determined to be 13 nM.



Scheme 1. Synthetic route of 2-aminothiazole derivatives and structure of **H154**.

†This paper is dedicated to Professor Eun Lee on the occasion of his honourable retirement.

Table 1. IC_{50} values of compound **H154** in various cancer cell lines

	IC_{50} (nM)					
	A549	SK-OV-3	SK-MEL2	HCT15	XF498	A431
H154	33.50	0.32	8.05	3.87	12.89	23.84
Doxorubicin	2.40	51.60	1.70	35.80	20.90	2.00

range of IC_{50} values.[8] In addition, pyrazole, keto-pyrrolopyrimidine, oxindole, isothiazole, thiazole analogs have been reported to be potent Trk inhibitors.[9] Although, some compounds have shown promise against prostate and pancreatic cancers[10] and in clinical trials,[11] these compounds inhibited a wide range of kinases or were selective among a short panel of kinases. However, when the inhibitory activity of compound **H154** was examined across the different 109 protein kinases at 10 μM, only three enzymes, Abl (53%), EphA1 (63%), and ZIPK (61%), were susceptible to **H154** with inhibition exceeding 50%. The other enzymes were unaffected, even at 10 μM. Moreover, kinase profiling showed that **H154** at 10 μM did not exhibit inhibitory activity against other tested receptor tysosine kinases, such as ALK, cKit, EGFR, FGFR, IR, PDGFR, Ret, Ron, Ros, and Tie2, indicating **H154** is a selective TrkA inhibitor.

In the past decade, it was reported that TrkA expression is increased in the late stage prostate cancers.[12] Recent literature also shows that the over-expression of TrkA is associated with many cancers including malignant melanomas,[13] ovarian carcinoma,[14] pancreatic cancer,[15] and colon cancer.[16] When the efficiency of the cytotoxic effects on various cells was examined,[17] compound **H154** exhibited anti-proliferation effects in the nanomolar concentration range, and more potency against ovary (SK-OV-3), melanoma (SK-MEL2) and colon (HCT15) tumor cells, which overexpress Trk receptors, as shown in Table 1. This indicates that the anti-proliferation effects of **H154** are due to the inhibition of TrkA.

After the discovery of the involvement of Trks in the biology of cancer, it was suggested that the inhibition of Trk kinases might be beneficial in a clinical oncology setting. The present data show that **H154** has selectively potent inhibitory activity against Trk receptor tyrosine kinase and has significant anti-tumor efficacy. These results suggest it could, therefore, serve not only as a useful research tool in the Trk-related investigations, but may also have therapeutic value as an anti-cancer agent in Trk-expressing tumors.

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7. TrkA kinase assay: A 15 μL solution with a final assay concentration of 250 μM substrate (KKKSPGEYVNIEFG) and approximately 10 ng of active *N*-terminal 6His-tagged recombinant human TrkA (residues 440-end, 41 kDa) in 8 mM MOPS (pH 7.0), 0.2 mM EDTA, 0.5% glycerol, 0.001% Brij-35, 0.01% 2-mercatoetnanol, and 1 mg/mL BSA was placed in a 96-well microtitier plate. The contents were mixed gently, and various concentrations of compound **H154** dissolved in DMSO were then added to the reaction mixture. After adding 10 μL of 25 mM magnesium acetate and 0.25 mM [γ-^{33}P]ATP(specific activity approximately 500-800 cpm/pmol), the reaction mixture was incubated for 10 minutes at 30 °C. The reaction was quenched by adding 5 mL 3% phosphoric acid and 10 μL aliquot transferred onto the appropriate area of a P30 filtermat. The filter mat was washed three times with 75 mM phosphoric acid for 5 minutes each, and once with methanol, and transferred to a sealable plastic bag. After adding 4 mL of a scintillation cocktail, the enzyme activity was read in a scintillation counter. The cpm of the enzyme sample with various concentrations of **H154** was compared with that of the control samples that containing all assay components plus 1 mL of 30% phosphoric acid.
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