Bioorganic & Medicinal Chemistry Letters 26 (2016) 5819–5824

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel substituted aminothiazoles as potent and selective anti-hepatocellular carcinoma agents

Huagang Lu [a], John Rogowskyj [a], Wenquan Yu [a], Anu Venkatesh [b], Noshena Khan [a], Shigeki Nakagawa [b], Nicolas Goossens [b], Anna P. Koh [b], Takaaki Higashi [b], Ganesh Gunasekaran [c], Myron E. Schwarz [c], Spiros P. Hiotis [d], Xiaodong Xu [a], William Kinney [a], Yujin Hoshida [b], Timothy Block [a], Andrea Cuconati [a,*], Yanming Du [a,*]

[a] Baruch S. Blumberg Institute, 3805 Old Easton Road, Doylestown, PA 18902, United States
[b] Division of Liver Diseases, Department of Medicine, Liver Cancer Program, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, United States
[c] Recanati/Miller Transplantation Institute, Icahn School of Medicine at Mount Sinai, United States
[d] Division of Surgical Oncology, Department of Surgery, Icahn School of Medicine at Mount Sinai, United States

ARTICLE INFO

Article history:
Received 30 August 2016
Revised 5 October 2016
Accepted 7 October 2016
Available online 10 October 2016

Keywords:
Hepatocellular carcinoma (HCC)
Substituted aminothiazole
Prodrug

ABSTRACT

Based on our previous identification of a disubstituted aminothiazole termed HBF-0079 with promising selective toxicity for HCC-derived cell lines versus non-HCC liver lines, a series of tri-substituted aminothiazole derivatives were prepared and evaluated. This work resulted in the discovery of isopropyl 4-(pyrazin-2-yl)-2-(pyrimidin-2-ylamino)thiazole-5-carboxylate, **14**, which displayed EC_{50} value of 0.11 μM and more than 450 times of selectivity, and its methyl carbonate prodrug **24** with improved solubility in organic solvents. Furthermore, **14**, was shown to reduce the proliferation of several liver cancer cells derived directly from patients.

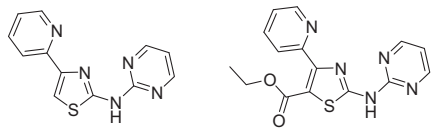
Primary cancer of the liver is a growing problem in the United States and the world. The most common adult primary liver cancer is hepatocellular carcinoma (HCC). This extremely deadly disease is currently one of the fastest growing cancers in incidence in the US.[1] It is the 5th most common cause of cancer in men and the 7th most common cause for cancer in women.[2] Primary liver cancer occurs predominantly in patients with liver cirrhosis, which can result from a variety of conditions that irritate the liver, such as chronic hepatitis C, hepatitis B,[3] or excessive alcohol consumption.[4]

HCC is traditionally treated by surgery or interventional ablative treatment.[5] However, due to the lack of biomarkers that detect tumors that are at an early enough stage, most patients are diagnosed when surgical resection is no longer feasible. Therefore, chemotherapy remains an important and necessary option for the treatment of inoperable HCC patients. There appears to be a diversity of molecular mechanisms underlying HCC development and maintenance. Microarray and proteomic studies have borne out evidence for molecular heterogeneity.[6,7] Based on the expression of specific genes, phenotypically identical tumors can be divided into six distinct profiles that correlate with different prognostic outcomes.[8,9] This heterogeneity suggests that a diversity of pathways are responsible for the establishment and progression of HCC tumors, requiring the structurally diverse anti-liver drugs with different action mechanisms. The current standard of care for patients with advanced forms of liver cancer is oral therapy with a multikinase inhibitor, Sorafenib.[10] This is the only anti-HCC drug approved by FDA and can improve median overall survival by approximately 3 months,[11] but its toxicity in normal liver cells has remained a concern because it is enacting an additional burden on the already diseased liver.[12]

In this report we describe our continued efforts toward the discovery of substituted aminothiazoles which are potent inhibitors of HCC and hepatoblastoma derived cells but are well tolerated by most other cancer-derived cell lines, as well as non-hepatic cell lines derived from non-cancerous tissue. There are only a handful of small molecules that have been reported to possess selective toxicity to liver cancer versus normal liver cells, making the aminothiazoles described here unusual.[13–19] Moreover, none of the other liver cancer-selective small molecules have been further progressed, to our knowledge. These compounds are mainly phenolic or flavonoid natural products which were isolated from herbs.[14–17] Using an orthogonal phenotypic screen,[20] we have identified a unique disubstituted aminothiazole, named

* Corresponding authors.
E-mail addresses: acuconati@arbutusbio.com (A. Cuconati), yanming.du@bblumberg.org (Y. Du).

http://dx.doi.org/10.1016/j.bmcl.2016.10.015
0960-894X/



HBF-0079
CC_{50}, Huh7, 1.1 μM
THLE2:Huh7, 35.450
PH5CH:Huh7, 40.294

IHVR-04042
CC_{50}, Huh7, 0.180 μM
THLE2:Huh7, 253.954
PH5CH:Huh7, 214.328

Figure 1. Potency and selectivity of HBF-0079 and IHVR-04042.

HBF-0079, which exhibits moderate micro-molar potency but remarkable selective toxicity for HCC-derived Huh7 cell lines but not non-HCC liver THLE-2 and PH5CH cell lines. It is also not toxic for most other cancer lines, further demonstrating it specificity for HCC. In addition, in an initial small set of structural alterations, we discovered that IHVR-04042 which possesses the incorporation of an ethyl ester substitution in the central thiazole ring of HBF-079, demonstrated a five-fold increased potency and five- to seven-fold increased selectivity (Fig. 1).[13] Therefore, more substituted aminothiazoles were synthesized and evaluated in vitro and ex vivo with a goal of increasing or maintaining potency and selectivity, and demonstrating efficacy in the real human HCC tissues.

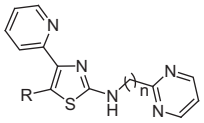
IHVR-04042 has a relatively flat/conjugated structure, centered by a thiazole with a pyridine substituted at 4-position and pyrimidine at 2-position connected via an amino linker. We began by evaluation of the replacement for ethyl ester group in the central thiazole ring of the IHVR-04042 and effect of an additional CH_2 between amino linker and right pyridine ring (Table 1). When the CH_2 was inserted, all the resulting compounds, **1**, **3**, **5**, **7** regardless of what types of substitutions at 5- position of the central thiazole ring, lost specific toxicities to the Huh7 cells (CC_{50} >40 μM and selectivities <2). Removal of the carbonyl group from the ester in IHVR-04042 provided **2** with a non-conjugated ether substitution, which displayed more than 10-fold weaker toxicity to Huh7 cells; when a bulky isopropyl ester was introduced, a 2-fold decrease in toxicity to Huh7 was observed while it was still selective to THLE2 and PH5CH cells with selectivities of 123.39 and 122.87 respectively; Insertion of one or two CH_2s between the carbonyl group and thiazole ring led to compound **6** and **8** with flexible pendant groups at 5-position of the thiazole, which exhibited 7- or 10-fold reduction of toxicity to Huh7 cells. Hydrolysis of the ester from compound **8** into acid yielded **9**, which further reduced the toxicity to the Huh7 cells. These results indicated that both ester at 5-position and pyrimidine to the amino linker were better to be connected directly.

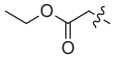
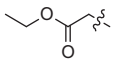
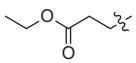
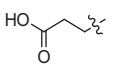
We next turned our attention to the pyridine at 4-position (Table 2) by replacing it with four other bioisosteres either with lower cLogP and basicity (compound **10**–**11**), lower basicity (compound **12**) or with a methyl group to increase the steric interaction with the ester group at 5-position of the thiazole so that to change the dihedral angle between pyridine and thiazole rings (compound **13**).[21] Interestingly, the position of an additional nitrogen to the pyridine ring resulted in different outcome. Pyrazine **10** is more potent than pyrimidine **11** despite both were less potent than IHVR-04042. Compound **12** with trifluoro substitution at para-position retained the potency to Huh7 but was more toxic to normal liver cells in THLE2 and PHCH. Incorporation of the methyl group at 5-position of the pyridine significantly decreased the potency suggesting this change is not favored.

As discussed above, isopropyl ester and several pyridine replacements can be tolerated at 5- and 4-positions of the thiazole moiety. We then assessed the combination of these two structural alterations (Table 3). It turned out when pyrazine was partnered with isopropyl ester, the resulting compound **14** demonstrated nearly 2-fold potency and selectivity increases while the two pyrimidine analogs, **15** and **16**, had similar potencies and selectivities as their ethyl ester analog **11**.

When treated with liver microsomes, **14** displayed a slightly improved stability in human liver microsomes, but more liability in mouse liver microsomes (Table 4). Detailed MetID analysis revealed that the hydrolysis of the ester and oxidation of the pyridine ring in IHVR-04042 were the major metabolisms that

Table 1
Toxicities toward Huh7 and selectivities compared to THLE2 and PH5CH cell lines with varying 5-substitution of thiazole and linker to the amino group



Cmpd	R	n	CC_{50} (μM) in Huh7	CC_{50} (μM) in THLE2	Selectivity (THLE2:Huh7)	CC_{50} (μM) in PH5CH	Selectivity (PH5CH:Huh7)
1	H	1	40.53 ± 10.27	49.57 ± 1.15	1.22	49.82 ± 0.41	1.23
2		0	3.19 ± 4.02	41.77 ± 10.84	13.08	39.04 ± 15.62	12.23
3		1	44.84 ± 10.64	49.65 ± 0.70	1.11	49.99 ± 0.01	1.12
4		0	0.41 ± 0.32	50.00 ± 0.00	123.39	49.79 ± 0.56	122.87
5		1	50.00 ± 0.00	50.00 + 0.00	1.00	50.00 ± 0.00	1.00
6		0	1.39 ± 0.94	50.00 ± 0.00	35.94	50.00 ± 0.00	35.94
7		1	49.38 ± 1.07	49.99 ± 0.02	1.01	50.00 ± 0.00	1.01
8		0	2.05 ± 0.95	10.47 ± 0.07	5.10	10.12 ± 0.70	4.93
9		0	14.14 ± 6.01	26.39 ± 4.61	1.87	17.68 ± 5.92	1.25