Published in final edited form as:

Bioorg Med Chem Lett. 2016 December 01; 26(23): 5819–5824. doi:10.1016/j.bmcl.2016.10.015.

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

Huagang Lu^a, John Rogowsky^{ja}, 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, and Yanming Du^a

^a, Baruch S. Blumberg Institute, 3805 Old Easton Road, Doylestown, PA 18902

^b, Division of Liver Diseases, Department of Medicine, Liver Cancer Program, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai

^c, Recanati/Miller Transplantation Institute, Icahn School of Medicine at Mount Sinai

^d, Division of Surgical Oncology, Department of Surgery, Icahn School of Medicine at Mount Sinai

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 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 its 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 CH₂ between amino linker and right pyridine ring (Table 1). When the CH₂ 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 (CC₅₀ > 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 CH₂s 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 occurred. While for **14**, the oxidation of pyridine was avoided due to the replacement with a pyrazine, however, the hydrolysis remained and oxidation of amino pyrimidine became significant (Figure 2).

The pharmacokinetics of **14** was investigated in mice and compared to IHVR-04042 (Table 5). Oral (po) bioavailability of **14** was 15%, a little bit higher than that of IHVR-04042 (10%). It is noteworthy that **14** had a high distribution volume (3.49 L/kg), indicating high propensity of tissue distribution. This was proved by the detection of **14** (33.0 ± 18.5 ng/g) in the liver after oral administration in Male CD-1 Mice (Table 5). This property should allow more active compound deposit in the liver and be beneficial for liver oriented diseases.

The compounds to date had been tested only with stable tissue culture lines which may not accurately reflect the behavior of primary cells derived directly from the organ. Therefore, having identified a more potent and selective lead compound, we decided to determine if this compound could reduce the proliferation of liver cancer cells derived directly from patients. Thus, compound **14**, which exhibited excellent selectivity for HCC derived cells in culture, was used to treat HCC tissues, ex vivo, from 16 patients. Surgically-resected fresh HCC tissues were sectioned into 300 μ m-thick slices and cultured with compound **14** at 1 μ M for 48h with media change every 24h, and compared to DMSO-treated control. The anti-tumor effect was assessed by the percentage (%) change of cell proliferation measured by Ki-67 immunostaining-positive cells calculated as an average of randomly chosen 5 high-power fields (Abcam). Among the tested patients, 5 patients showed reduction of the staining (i.e., response rate of 38%) as shown in Figure 2 together with representative Ki-67 staining comparing the drug-treated with control tissues (each patient is denoted by anonymous ex vivo number). It is not clear why only 38% of the tumors yielded IHVR-14 –35–119 sensitive proliferation, but it is not unusual for explants to behave heterogeneously to drugs. Effort is under way, in other work, to determine if expression and genetic analysis of the sensitive and resistant cultures can reveal clues.

Despite the increased potency and selectivity, similar to IHVR-04042, compound **14** had poor solubility in most of the organic solvents and water (6.35 μ M at PH 4 and 5.20 μ M at PH 7.4, table 4). This prompted us to explore prodrug approach to get this molecule into a solution.²² Based on the structure of our lead compound **14**, the amino linker was selected to attach three prodrug moieties for assessment, including methyl phosphate,²³ methyl carbonate²⁴ and methyl ether, which can change the physical chemical property of the parent compound and have been used to enhance the solubility of some insoluble compounds

(Table 6). As expected, all four prodrugs now can be easily dissolved in some organic solvents, such as methylene chloride, ethyl acetate, ethanol, and this improvement can greatly facilitate the preparation of higher concentration formulation. Di-tert-butyl methyl phosphate is an interesting prodrug carrier. In ester form, it can help to improve solubility in organic solvents; while it is hydrolyzed to acid form, it can increase the solubility in water. Incorporation of this moiety to compound **4** yielded compound **21**, which retained potency and selectivity; On the contrary, when this group was added to **14** to have **22**, the potency dropped for about 9-fold. The difference may be attributed to inefficient hydrolysis to release the parent **14**. This may explain why prodrug **23** did not show any selectivity when a more difficult to remove methyl ether was used. However, when methyl carbonate was used to modify the compound **14**, the resulting prodrug **24** exhibited an even slightly better potency and selectivity than the parent compound **14**, indicating a better permeability was achieved. Thus, through this round of selection, two prodrug moieties were found that could either retain or increase the potency and selectivity of the parent compounds. Compound **24** offered improvements in potency, selectivity, and solubility in organic solvents. Therefore, it was chosen for further studies, for which data will be reported in due course.

The synthesis of this novel series of substituted amino thiazoles was achieved through condensation of a thiourea with a bromo-ketone. This route is efficient and versatile in that it allows us to introduce different substitution groups for evaluation. As exemplified for the synthesis of **14**, nucleophilic substitution of isopropyl acetate to methyl pyrazine-2-carboxylate **25** provided a β -keto ester **26**, which was brominated at alpha position through the reaction with phenyltrimethylammonium tribromide (PMAT) or bromine to afford a key intermediate **27**. The other key intermediate **28** was prepared following a reference method.²⁵ Condensation between **27** and **28** delivered **14**. Further alkylation of **14** with chloromethyl isopropyl carbonate was accomplished under basic condition to form the corresponding prodrug **24**. Detailed synthetic information for other intermediates and products can be found in the supporting information.

In summary, a series of substituted aminothiazoles were designed. Multiple rounds of the optimization were carried out to improve cell based potency and selectivity. This resulted in the identification of compound **14** with a favorable efficacy in the several human HCC isolates tissues and its prodrug **24** with improved solubility in organic solvents and potential for further development.

Acknowledgement

This project is supported by Pennsylvania Department of Health through the Commonwealth Universal Research Enhancement (CURE) program (PA grant, 4100059194). De-identified surgical HCC tissue acquisition was supported by Mount Sinai Biorepository. We also acknowledge the supports from Uehara Memorial Foundation for Shigeki Nakagawa, Japan Society for the Promotion of Science for Takaaki Higashi, FLAGS foundation, Nuovo-Soldati Cancer Research Foundation, an advanced training grant from Geneva University Hospital for Nicolas Goossens, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health DK099558, Irma T. Hirschl Trust, European Union ERC-AdG-2014 HEPCIR for Yujin Hoshida. This study was also partially supported by the Hepatitis B Foundation and the Commonwealth of Pennsylvania. The authors also thank Dr. Chunyan Han of Pharmaron for discussion of metabolite ID results.

References

1. El-Serag HB Gastroenterol. 2004, 127, S27.
2. Fatima S; Lee NP; Luk JM World J Clin Oncol. 2011, 2, 311. [PubMed: 21876852]
3. Sene WR; Zhang Y; Chen Y ISRN Oncol. 2012, 1.
4. Bosetti C; Levi F; Lucchini F; Zatonski WA; Negri E, La VC J Hepatol 2007, 46, 827. [PubMed: 17336419]
5. Colombo M; Sangiovanni A Liver Int. 2015, 35, 129. [PubMed: 25529098]
6. Thorgeirsson SS; Lee JS; Grisham JW Hepatology, 2006, 43, S145. [PubMed: 16447291]
7. Thorgeirsson SS; Lee JS; Grisham JW, J Hepatol. 2006, 44, 798. [PubMed: 16488507]
8. Boyault S; Rickman DS; Reyniès A; Balabaud C; Rebouissou S; Jeannot E; Hérault A; Saric J; Belghiti J; Franco D; Bioulac-Sage P; Laurent-Puig P; Zucman-Rossi J Hepatology, 2007, 45, 42. [PubMed: 17187432]
9. Goossens N; Sun X; Hoshida Y Hepat Oncol. 2015, 2, 371. [PubMed: 26617981]
10. Peck-Radosavljevic M Liver Cancer 2014, 3, 125. [PubMed: 24945003]
11. Llovet JM; Ricci S; Mazzaferro V; Hilgard P; Gane E; Blanc JF; de Oliveira AC; Santoro A; Raoul JL; Forner A; Schwartz M; Porta C; Zeuzem S; Bolondi L; Greten TF; Galle PR; Seitz JF; Borbath I; Haussinger D; Giannaris T; Shan M; Moscovici M; Voliotis D; Bruix J N. Engl. J. Med 2008, 359, 378e390.
12. Teo YL; Ho HK; Chan A Cancer Treat. Rev 2013, 39, 199. [PubMed: 23099278]
13. Cuconati A; Mills C; Goddard C; Zhang X; Yu W; Guo H; Xu X; Block TM, PLOS ONE, 2013, 8, e54595. [PubMed: 23355882]
14. Susanti S; Iwasaki H; Itokazu Y; Nago M; Taira N; Saitoh S; Oku H J. Nat. Med 2012, 66, 614. [PubMed: 22350142]
15. Yuan L; Wang J; Xiao H; Xiao C; Wang Y; Liu X Toxicol. Appl. Pharmacol, 2012, 265, 83. [PubMed: 23026832]
16. Jeon Y-M; Kim J-G; Lee J-C Nat. Prod. Sci 2004, 10, 129.
17. Gao H; Wang H; Peng J Cell Biochem. Biophys 2014, 69, 27. [PubMed: 24068521]
18. Al-Qubaisi M; Rozita R; Yeap S-K; Omar A-R; Ali A-M; Alitheen NB Mol, 2011, 16, 2944.
19. Ho HK; Chua BT; Wong W; Lim KS; Teo V; Ong H-T; Chen X; Zhang W; Hui KM; Go ML; Ullrich A Mol. Oncol 2014, 8, 1266. [PubMed: 24839937]
20. Swinney DC; Anthony J Nat. Rev. Drug Discov 2011, 10, 507. [PubMed: 21701501] This paper revealed that based on recent statistical analysis more of the first-in-class drugs with novel mechanisms of action come from phenotypic screening. Among all the 50 first-in-class small molecule drugs approved between 1999 and 2008, 28 came from phenotypic screening, while 17 were from target-based approaches.
21. Jean DJ, St.; Fotsch C J. Med. Chem 2012, 55, 6002. [PubMed: 22533875]
22. Rautio J; Kumpulainen H; Heimbach T; Oliyai R; Oh D; Järvinen T; Savolainen J Nat. Rev. Drug Discov 2008, 7, 255.
23. Kadow JF; Ueda Y; Meanwell NA; Connolly TP; Wang T; Chen C-P; Yeung K-S; Zhu J; Bender JA; Yang Z; Parker D; Lin P-F; Colonno RJ; Mathew M; Morgan D; Zheng M; Chien C; Grasela DJ Med. Chem 2012, 55, 2048.
24. Blumberg LC; Remenar JF; Almarsson O; Zeidan TA, PCT Int. Appl, 2010151711.
25. Cuconati A; Xu X; Block TM PCT Int. Appl, 2013052613.

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

Figure 2.
Major Metabolites of 14 and IHVR-04042 from incubation with liver microsomes

Figure 3.
Anti-cell proliferative effect of compound 14 in ex vivo culture of clinical HCC tissues.

Scheme 1.
Synthesis of compound 24

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

Cmpd	R	n	CC ₅₀ (μM) in Huh7	CC ₅₀ (μM) in THLE2	Selectivity (THLE2:Huh7)	CC ₅₀ (μM) in PH5CH	Selectivity (PH5CH:Huh7)
9		0	14.14 ± 6.01	26.39 ± 4.61	1.87	17.68 ± 5.92	1.25

Table 2.

Evaluation of pyridine replacement

Cmpd	R	CC ₅₀ (μM) in Huh7	CC ₅₀ (μM) in THLE2	Selectivity (THLE2:Huh7)	CC ₅₀ (μM) in PH5CH	Selectivity (PH5CH:Huh7)
10		0.59 ± 0.18	47.08 ± 8.77	80.06	50.00 ± 0.00	85.03
11		1.60 ± 0.68	40.78 ± 13.04	25.46	39.98 ± 17.35	24.97
12		0.17 ± 0.08	14.99 ± 7.11	90.32	9.34 ± 2.16	56.24
13		46.12 ± 5.58	49.15 ± 1.69	1.07	50.00 ± 0.00	1.08

Table 3.

Evaluation of pyridine replacement in presence of isopropyl ester

Cmpd	R	CC ₅₀ (μM) in Huh7	CC ₅₀ (μM) in THLE2	Selectivity (THLE2:Huh7)	CC ₅₀ (μM) in PH5CH	Selectivity (PH5CH:Huh7)
14		0.11 ± 0.01	50.00 ± 0.00	471.70	50.00 ± 0.00	471.70
15		1.21 ± 0.41	46.20 ± 5.74	38.11	50.00 ± 0.00	41.24
16		1.62 ± 0.87	50.00 ± 0.00	30.96	50.00 ± 0.00	30.96

Table 4.

Aqueous solubility and metabolic stability of 14 compared with IHVR-04042

cmpd	Solubility (μM)		Remaining in LM (area %) @ 60 min in MetID test	
	PH = 4.0	PH = 7.4	Human	Mouse
04042	112.84	23.84	63.1	43.32
14	6.35	5.20	70.91	36.17

Table 5.

Pharmacokinetics profile of 14 compared with IHVR-04042

Cmpd	Route	Dose (mg/kg)	T _{max} (hr)	C _{max} (ng/ml)	T _{1/2} (hr)	V _{ss} (L/Kg)	AUC _{last} (hr*ng.ml)	Liver Tissue Conc. (ng/g)	Bioavailability (%)
04042	iv	2	0		0.24	1.45	224		
	po	10	0.58	94.1	1.91		19.5		10
14	iv	1	0		0.44	3.49	142	ND	
	po	2.5	0.44	48.7	ND		21.6	33.0 ± 18.5	15.2

Table 6.

Evaluation of three types of prodrugs

Cmpd	A	R	CC ₅₀ (μM) in Huh7	CC ₅₀ (μM) in THLE2	Selectivity (THLE2:Huh7)	CC ₅₀ (μM) in PH5CH	Selectivity (PH5CH:Huh7)
21			0.38 ± 0.22	42.23 ± 7.77	111.42	50.00 ± 0.00	131.93
22			0.98 ± 0.39	50.00 ± 0.00	51.28	50.00 ± 0.00	51.28

Cmpd	A	R	CC ₅₀ (μM) in Huh7	CC ₅₀ (μM) in THLE2	Selectivity (THLE2:Huh7)	CC ₅₀ (μM) in PH5CH	Selectivity (PH5CH:Huh7)
23			50.00 ± 0.00	50.00 ± 0.00	1.00	50.00 ± 0.00	1.00
24			0.095 ± 0.059	50.00 ± 0.00	528.17	50.00 ± 0.00	528.17