Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Aza follow-ups to BI 207524, a thumb pocket 1 HCV NS5B polymerase inhibitor. Part 1: Mitigating the genotoxic liability of an aniline metabolite

Pierre L. Beaulieu [a,*], Gordon Bolger [b], Martin Duplessis [a], Alexandre Gagnon [a], Michel Garneau [b], Timothy Stammers [a], George Kukolj [b], Jianmin Duan [b]

[a] Medicinal Chemistry Department, Boehringer Ingelheim (Canada) Ltd, Research and Development, 2100 Cunard Street, Laval, Quebec H7S 2G5, Canada
[b] Biology Department, Boehringer Ingelheim (Canada) Ltd, Research and Development, 2100 Cunard Street, Laval, Quebec H7S 2G5, Canada

ARTICLE INFO

Article history:
Received 26 October 2014
Revised 6 December 2014
Accepted 9 December 2014
Available online xxxx

Keywords:
Hepatitis C virus
NS5B polymerase
Antiviral
Genotoxicity
Ames

ABSTRACT

A series of heterocyclic aza-analogs of BI 207524 (**2**), a potent HCV NS5B polymerase thumb pocket 1 inhibitor, was investigated with the goal to reduce the liability associated with the release of a genotoxic aniline metabolite in vivo. Analog **4**, containing a 2-aminopyridine aniline isostere that is negative in the Ames test was identified, and was found to provide comparable GT1a/1b potency to **2**. Although the cross-species PK profile, poor predicted human liver distribution of analog **4** and allometry principles projected high doses to achieve a strong antiviral response in patients, this work has provided a path forward toward the design of novel thumb pocket 1 NS5B polymerase inhibitors with improved safety profiles.

In the last years, significant breakthroughs have occurred in the treatment and cure of disease conditions associated with chronic hepatitis C virus infection (HCV). The first Direct Acting Antivirals (DAAs) that enhance efficacy and tolerability of pegylated-interferon/ribavirin based therapies have reached the market and are providing patients with sustained viral responses (SVR) and cure in up to 80–90% of genotype 1 (GT1) treatment naïve patients.[1] More recently, IFN-free regimens combining two or three DAAs with complementary modes of actions have shown potential to further improve cure rates (SVR >90%) and tolerability and are showing improved efficacy in the harder-to-treat GT1a and cirrhotic populations.[2,3] To this end, we have been pursuing allosteric inhibitors of the HCV NS5B polymerase as partners in IFN-free combination therapy (e.g., with protease inhibitor faldaprevir).[4]

We have described the discovery and progression to the clinic of indole-based inhibitors that bind to the thumb pocket 1 allosteric site of NS5B and prevent inter-domain interactions between the thumb and finger regions of the protein, resulting in a replication-deficient enzyme. Proof of concept for this class of inhibitors was achieved with BILB 1941 (**1** Fig. 1) that produced up to 2.5 $\log_{10}$ reductions in viremia in HCV GT1 infected patients but was discontinued because of gastrointestinal intolerance at doses required to maintain a strong antiviral response (>450 mg TID).[5] BI 207524 (**2**) is a follow-up compound from this class with improved potency and a cross-species PK profile consistent with achieving a robust antiviral response at a reduced dose (400 mg BID), based on our recently published liver-corrected inhibitory quotient model (LCIQ).[6] In this model, inhibitor liver C_{trough} concentrations corresponding to $\geqslant$500-fold the EC_{50} are predicted to provide a strong antiviral response in infected patients.[7]

During preclinical development of **2**, a genotoxic aniline metabolite (**3**, X = Y = Z = CH) was detected in both human and rat liver microsome (~1 ppm/min in HLM in an NADPH-independent manner) and simulated gastric fluid (SGF) incubation studies and in vivo in rats, thus compromising further progression of this candidate.[6] A well established medicinal chemistry strategy to address such a liability consists of replacing the aniline with isosteres (e.g., heterocycles) that can modulate the binding affinity of the aromatic amine to the CYP1A2 enzyme cavity and stabilize anionic forms implicated in the formation and stability of the reactive nitrenium ion that is formed upon metabolic activation.[8] The suitability of the replacements can be assessed using computational methods and verified by testing in the Ames test. One of the strategies implemented in our program to resolve the genotoxic liability of **2**, involved replacement of the right-hand-side 4-amino-2-ethoxycinnamic acid moiety in **2** by nitrogen-containing isosteres

* Corresponding author.

http://dx.doi.org/10.1016/j.bmcl.2014.12.028
0960-894X/

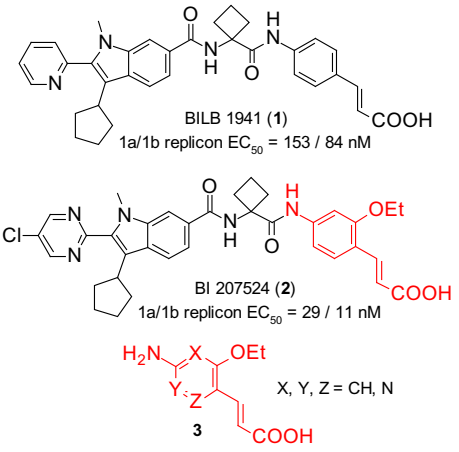


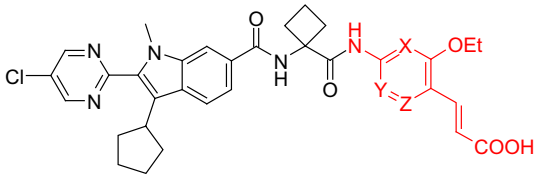
Figure 1. HCV NS5B polymerase thumb pocket 1 leads.

3 that unlike the parent aniline, were determined in silico to be devoid of genotoxic potential.[9]

The isosteric aza-BI 207524 analogs investigated in this study are listed in Table 1 and the synthesis of required right-hand-side heterocyclic aniline replacements is described in Scheme 1. For inhibitor **4**, 2-amino-6-bromopyridine **10** was treated with KO*t*Bu/EtOH and regioselectively iodinated to provide 5-iodopyridine **11** which was converted to 4-aminocinnamate **12** using a standard Heck Pd-catalyzed cross-coupling protocol with ethyl acrylate. Fragment **12** was then condensed with 1-aminocyclobutane carboxylic acid chloride hydrochloride and converted to inhibitor **4** as previously described.[6] 4-Ethoxypyridine *N*-oxide **13** was converted to 2-amino-4-ethoxypyridine **14** which was then iodinated and cross-coupled to ethyl acrylate as described for **12**, to provide building block **15** that was converted to inhibitor **5**. 2,3-Dihydroxypyridine **16** was converted to nitropyridine **17** and then to 2-amino-5-chloropyridine **18** as described in the literature for the corresponding methoxy analog.[10] Heck cross-coupling with *tert*-butylacrylate under the usual conditions provided cinnamate **19** that was used to prepare inhibitor **6**. Commercially available 2-aminopyrimidine **20** was acetylated and chlorinated to provide 4-chloropyrimidine **21**. Treatment with sodium ethoxide resulted in simultaneous deprotection of the amine function and displacement of the chloro group with ethoxy. The ester functionality was subsequently reduced to the corresponding benzylic alcohol that was then oxidized to provide aldehyde **22**. Aldehyde **22** was converted to cinnamate **23** using a Horner–Wadsworth–Emmons condensation and elaborated to inhibitor **7**. Chloropyridazine **24** was prepared in four steps following a literature procedure.[11] The ester function was reduced to the corresponding aldehyde using DIBAL which was homologated to cinnamate ester **25** through a Horner–Wadsworth–Emmons condensation. The chlorine atom was converted to an amine by sequential azide displacement and a two step reduction using triphenylphosphine followed by hydrolysis to give **26**. Aminopyridazine **26** was converted to analog **8** in the usual manner. Finally, 2-amino-6-chloropyrazine **27** was ethoxylated and iodinated under previously described conditions to provide iodopyrazine **28** that was converted to cinnamate **29** and subsequently to inhibitor **9**.

BI 207524 (compound **2**), was initially considered as an attractive follow-up candidate to BILB 1941 since it combined improved potency (7 to 8-fold; achieved through decreasing the measured acidity of the carboxyl function by introduction of the conjugated ethoxy group) with a predicted human PK profile consistent with lower dosing requirements (400 mg BID to achieve a liver-corrected IQ = 500, predictive of a strong antiviral response).[6] The success of our genotoxicity mitigating strategy relied on the identification of compounds that would maintain or improve potency relative to **2** (particularly against the less sensitive GT1a) as well as retain its favorable PK profile. Results from biological testing of BI 207524 and six aza-analogs are shown in Table 1.[12] In a polymerase inhibition assay using a C-terminally truncated NS5BΔ21 construct,[13] all compounds had comparable potency to **2** (IC_{50} = 60–110 nM), suggesting that introduction of nitrogen atoms in the aniline moiety was neither detrimental nor beneficial to interactions with the enzyme binding site. In a cell-based

Table 1
BI 207524 and aza-analogs containing non-genotoxic aniline replacements



Entry	X	Y	Z	IC_{50}[a] (nM)	GT1b EC_{50}[b] (nM)	GT1a EC_{50}[b] (nM)	TC_{50} (MTT, μM)	HLM/RLM[c] $t_{1/2}$ (min)	Caco-2[d] $\times 10^{-6}$ (cm/s)	Rat PK $C_{1h/2h}$[e] or C_{max}[f] (μM)	Liver/plasma ratio[h]	Log*D* (pH 7.4)
2	CH	CH	CH	84	13/11	29	38	102/104	13	3.2[g]	5.5 (6 h)	4.1
4	N	CH	CH	85	15/14	27	56	66/111	15	5.9[f]	2	>5.9
5	CH	N	CH	85	33/—	—	>10	145/104	15	1.8[f]	—	3.0
6	CH	CH	N	110	20/—	—	>10	275/143	7.5	0.4/0.1[e]	5	3.9
7	N	N	CH	63	40/—	89	>10	239/>300	4.3	0.1/0.03[e]	—	—
8	CH	N	N	80	360/—	—	>10	136/227	1.6	—	—	3.4
9	N	CH	N	100	20/—	34	>10	146/>300	13	2.1/0.9[e]	5	3.5

[a] GT1b NS5BΔ21 ($n \geqslant 2$).
[b] Luciferase reporter and RT-PCR replicon assay values are reported; see Refs. 6 and 13 ($n \geqslant 2$).
[c] Human and rat microsomal stability at 2 μM initial concentration.
[d] Apical to basolateral permeability.
[e] Following oral administration to rats as mixtures of four compounds at a dose of 4 mg/kg each in 0.5% Methylcellulose and 0.3% Tween-80 + 1% *N*-methylpyrrolidone (NMP).[14]
[f] Administered orally as a single compound at a dose of 5 mg/kg in 0.5% Methylcellulose and 0.3% Tween-80 + 1% *N*-methylpyrrolidone (NMP).
[g] Normalized to 5 mg/kg from a 10 mg/kg dose.
[h] Determined at 2 h for cassettes or 8 h for single compound PK.[14]

Please cite this article in press as: Beaulieu, P. L.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2014.12.028