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Structure-based design of novel HCV NS5B thumb pocket 2 allosteric inhibitors with submicromolar gt1 replicon potency: Discovery of a quinazolinone chemotype

Pierre L. Beaulieu [a,*], René Coulombe [a], Jianmin Duan [b], Gulrez Fazal [a], Cédrickx Godbout [a], Oliver Hucke [a], Araz Jakalian [a], Marc-André Joly [a], Olivier Lepage [a], Montse Llinàs-Brunet [a], Julie Naud [a], Martin Poirier [a], Nathalie Rioux [b], Bounkham Thavonekham [a], George Kukolj [b], Timothy A. Stammers [a]

[a] Medicinal Chemistry, 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

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ABSTRACT

We describe the structure-based design of a novel lead chemotype that binds to thumb pocket 2 of HCV NS5B polymerase and inhibits cell-based gt1 subgenomic reporter replicons at sub-micromolar concentrations (EC_{50} <200 nM). This new class of potent thumb pocket 2 inhibitors features a 1*H*-quinazolin-4-one scaffold derived from hybridization of a previously reported, low affinity thiazolone chemotype with our recently described anthranilic acid series. Guided by X-ray structural information, a key NS5B–ligand interaction involving the carboxylate group of anthranilic acid based inhibitors was replaced by a neutral two-point hydrogen bonding interaction between the quinazolinone scaffold and the protein backbone. The in vitro ADME and in vivo rat PK profile of representative analogs are also presented and provide areas for future optimization of this new class of HCV polymerase inhibitors.

Inhibitors of the virally-encoded NS5B RNA-dependent RNA polymerase of the hepatitis C virus have acquired a prominent position in the development of more effective and better-tolerated interferon-free therapies for the treatment of chronic HCV infection. The addition of 1st generation NS3/4A protease inhibitors to the pegylated-interferon-α/ribavirin (PEG-IFN/RBV) standard of care (SoC) has increased sustained virological response (SVR) to 68–75% and shortened duration of treatment in genotype (gt) 1 patients.[1] However, interferon-free regimens consisting of multiple combinations of Direct Acting Antivirals (DAAs) with complementary mechanisms of action and RBV, have shown similar SVR rates with increased tolerability compared to the newly introduced SoC (IFN + RBV + PI).[2] More recently, triple DAA combinations have shown potential to increase SVR rates to >90% in the gt1 population.[3] A key element of these new promising interferon-free therapies is the use of nucleoside (tide) or allosteric non-nucleoside NS5B inhibitors in combination with NS3/4A or NS5A inhibitors.

Over the last years, we have been investigating the use of thumb pocket 1 NS5B allosteric inhibitors in combination with the protease inhibitor faldaprevir.[4] BI 207127 (Fig. 1) is a potent inhibitor of gt1–6 replicons (EC_{50} = 11−98 nM).[5] The IFN-free combination of faldaprevir + BI 207127 + RBV demonstrated high efficacy and good tolerability in treatment-naïve patients with 16–28 weeks of treatment achieving overall SVR rates up to 69% (up to 85% in gt1b) and is currently being evaluated in phase 3 clinical trials for the treatment of gt1b HCV chronic infection.[6]

To augment our efforts in the development of IFN-free regimens based on the combination of faldaprevir with non-nucleoside NS5B thumb pocket 1 inhibitors we recently reported the discovery of anthranilic acid derivatives (e.g., compounds **1** and **2**, Figs 1 and 2) that bind to 'thumb pocket 2', a spatially distinct allosteric site on the polymerase situated at the base of the thumb domain, some 30 Å away from the enzyme active site.[7,8] Similarly to previously reported clinical candidates that bind to this allosteric pocket (e.g., filibuvir and lomibuvir; Fig. 1)[9] anthranilic acid-based inhibitors are structurally distinct from indole-based thumb pocket 1 compounds (e.g., BI 207127) and were shown to exhibit additive potency and distinct resistance profiles with the later. Anthranilic acid amides such as **1** possessed comparatively promising antiviral potencies in both gt1a and gt1b replicons (RT-PCR EC_{50} ~150 nM) and optimization of these acid derivatives was actively pursued toward the identification of more potent analogs with suitable qualifications for preclinical development. In a parallel effort we also pursued structure-based design aiming to increase chemical

* Corresponding author. Tel.: +49 (6132) 77-3092.
E-mail address: resgeneral.lav@boehringer-ingelheim.com (P.L. Beaulieu).

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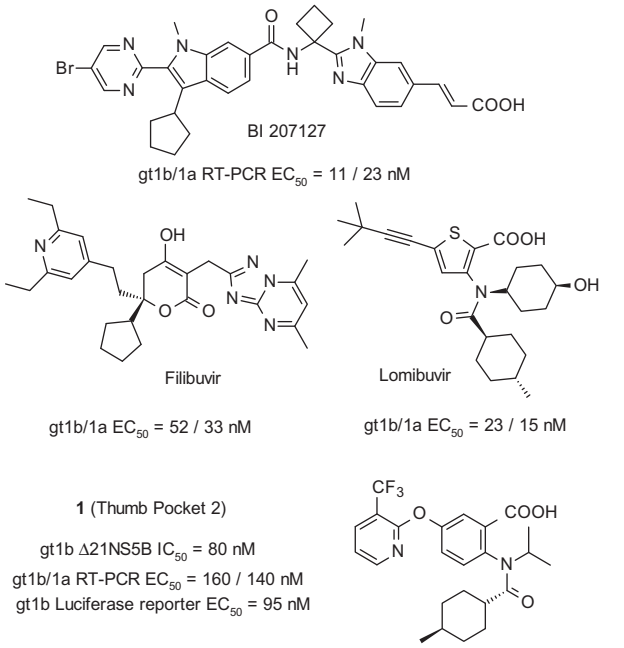

Figure 1. Structure of NS5B thumb pocket 1 and 2 clinical candidates and thumb pocket 2 lead.

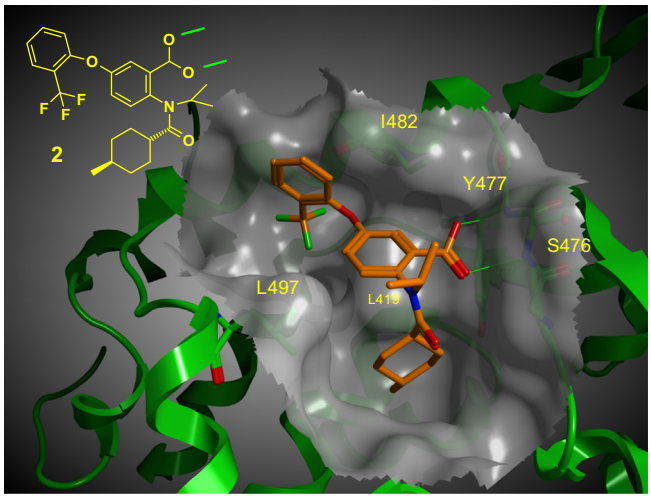

Figure 2. X-ray structure of a complex depicting key interactions between anthranilic acid derivative **2** and NS5B polymerase (PDB 4JJS).

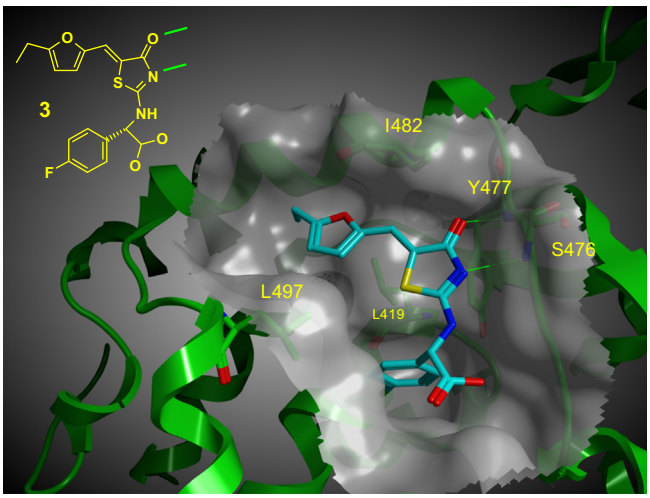

Figure 3. X-ray structure of thumb pocket 2 thiazolone **3** (PDB 2HWI) in complex with NS5B polymerase.

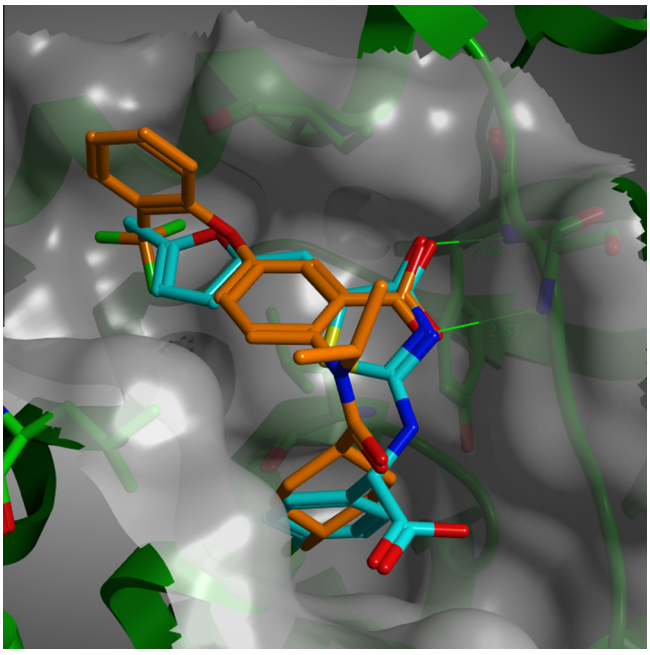

Figure 4. Overlap of NS5B complexes with compounds **2** and **3** showing conservation of key inhibitor–protein interactions.

diversity by replacing the carboxylic acid function of **1** by non-ionizable surrogates in order to identify inhibitors with distinct ADME-PK profiles and potential for addressing possible liabilities often associated with carboxylic acid containing molecules such as reduced cell permeability and the formation of reactive metabolites (e.g., acylglucuronides). The discovery of a novel NS5B thumb pocket 2 chemotype based on a neutral 1*H*-quinazolin-4-one scaffold is described herein.

The X-ray crystal structure of anthranilic acid derivative **2** (Δ21 NS5B IC_{50} = 0.20 μM/gt1b luciferase replicon EC_{50} = 0.64 μM), a close analog of **1**, in complex with NS5B polymerase is shown in Figure 2.[7b,10]

A key inhibitor–protein interaction that is characteristic of anthranilic acid inhibitors such as **2** is the two-pronged hydrogen bond highlighted in Figure 2 between the carboxyl group of the inhibitor and the backbone NHs of Y477 and S476. Other notable contacts include the lipophilic 4-methylcyclohexyl and trifluoromethyl groups of the inhibitor that bind to hydrophobic pockets on the enzyme. The *N*-isopropyl group points toward solvent and serves to orient the cyclohexyl amide moiety towards a well-defined binding pocket. The remainder of the molecule (i.e., the benzene ring scaffold) lies flat between the two walls of an extended protein channel, resting on the side chain of L419 and enclosed on each side by L497 and I482 that in part constitute the opposing walls of the pocket.

Our design of non-acidic inhibitors had to consider the critical interactions mediated by the carboxylic acid group of **1** or **2** and the proximal protein backbone residues. Not surprisingly, isosteric replacements for the COOH group were not well tolerated, presumably because they require significant protein conformational adaptation to accommodate larger groups (unpublished results). Several years ago, Valeant Pharmaceuticals reported a series of