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Discovery of substituted *N*-phenylbenzenesulphonamides as a novel class of non-nucleoside hepatitis C virus polymerase inhibitors

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ABSTRACT

The RNA-dependent RNA polymerase NS5B of the hepatitis C virus (HCV) has emerged as one of the key targets for antiviral drug discovery. Here we describe a novel non-nucleoside inhibitor (NNI) chemotype identified by screening: The substituted *N*-phenylbenzenesulphonamides (SPBS) which showed reversible inhibition of NS5B from HCV genotype 1b with IC_{50} values up to 40 nM. Based on the decreased inhibitory activity against a recombinant NS5B protein carrying the mutation L419M or M423T we assumed that the SPBS inhibitors bind to the thumb site II which has already been described as the allosteric binding site for the NNI carboxy thiophene. The postulated binding site was consequently confirmed by solving two co-crystal structures of NS5B in complex with SPBS analogues at 2.3 and 2.2 Å resolutions. The inhibitors are hydrogen-bonded to the main chain Ser476 and Tyr477 and to the side chain of Arg501. In addition, the inhibitors displayed van der Waals interactions with several residues of the hydrophobic binding pocket Leu419, Ile482, Leu497, Met423 and Trp528. Notably, the two SPBS analogues reported here revealed significant differences in addressing the NH-group of the main chain Tyr477 by hydrogen-bonds, water-mediated or directly, which provoked a shift of the carboxyphenyl group of the inhibitors towards the His475 position for the water-mediated binding mode. Interestingly, the differences observed in the binding mode led to a different cross resistance profile at positions M423 and I482. Using a panel of 38 individual NS5B proteins derived from different HCV genotypes, we could demonstrate inhibitory activity of the SPBS against polymerases from HCV genotypes 1a and 1b whereas the inhibitor class failed to inhibit any of the non-genotype 1 polymerases efficiently. Furthermore we demonstrated initial antiviral activity for SPBS against the subgenomic replicons of HCV genotypes 1a and 1b, respectively, and no considerable cytotoxic potential against a panel of ten different cell types.

1. Introduction

Three percent of the world's population is infected with the hepatitis C virus (HCV), an enveloped 9.6 kb positive-sense single-stranded RNA virus belonging to the *Flaviviridae* family. In 70% of cases the infection becomes chronic with the risk of developing liver cirrhosis and hepatocellular carcinoma (Hoofnagle, 2002). The standard treatment of HCV infection, a combination of pegylated interferon alpha (pegIFN-α) and ribavirin (RBV), is associated with severe side effects and the response rate is unfortunately low for individuals infected with genotype 1, the most prevalent strain in North America, Europe and Asia. Therefore a high unmet medical need exists for the development of safer and more effective drugs. Direct-acting antivirals (DAA), currently investigated in clinical trials for the so called specifically targeted antiviral therapy of HCV (STAT-C), mainly focus on three molecular targets: the NS3/4A serine protease, the NS5A phosphoprotein, and the NS5B polymerase. Fortunately, very recently the first two DAAs (Telaprevir and Boceprevir) have been approved by the US Food and Drug Administration for the treatment of patients infected with HCV. NS5B, an RNA-dependent RNA polymerase, is structurally organised in a typical 'right hand' polymerase shape with finger, palm, and thumb subdomains surrounding a completely encircled active site (Bressanelli et al., 1999; Lesburg et al., 1999). The RNA-dependent RNA polymerase activity with no counterpart activity in humans is strictly essential for viral replication (Behrens et al., 1996; Kolykhalov et al., 2000) which makes NS5B an attractive antiviral drug target. HCV polymerase inhibitors can be classified into two major categories, the nucleoside analogue

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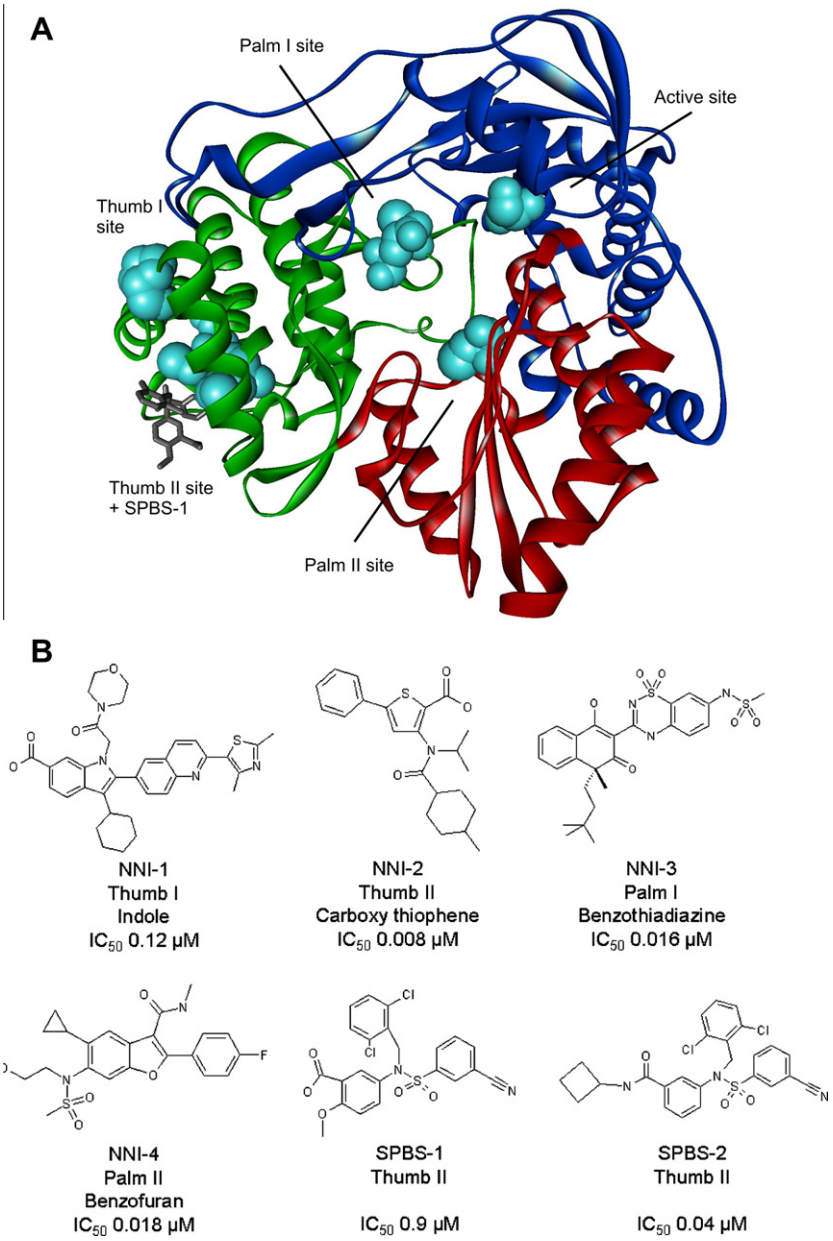

Fig. 1. 3D structure of HCV polymerase NS5B and structural formula of NNIs used in this study. (A) Palm, finger and thumb subdomain of NS5B are coloured in red, blue and green, respectively. Residues P495 (thumb site I), L419 and M423 (thumb site II), Q446 and C451 (palm site I), C316 (palm site I) and S282 (active site) associated in NNI or NI resistance are shown as light blue spheres. SPBS-1 bound to thumb site II is shown in grey. (B) For the NNI reference inhibitors (Bosse et al., 2008; Botyanszki et al., 2006; Chan et al., 2004b; Kneteman et al., 2009) and SPBS analogues designated as SPBS-1 and SPBS-2 the respective binding site is given below the structure. IC_{50} values have been determined in biochemical primer-dependent transcription assay on HCV genotype 1b polymerase (GeneBank CAB10747).

inhibitors (NIs) which act as chain terminators and bind to the active site and the non-nucleoside inhibitors (NNIs) which bind to one of at least four different allosteric binding sites outside the active site. In the last years several NNI scaffolds have been described targeting one of these binding pockets, namely the indoles which bind to the thumb site I also known as thumb-finger site or NNI-1 site (Botyanszki et al., 2006), the thiophenes which bind to the thumb site II (NNI-2 site) Wang et al., 2003, the benzothiadiazines which bind to the palm site I (NNI-3 site) (Bosse et al., 2008) and finally the benzofurans which bind to the palm site II (NNI-4) Howe et al., 2008 (Fig. 1).

It is anticipated that the emergence of viral resistance is likely to limit the use of NNIs (and all other DAAs) as monotherapy and that the future HCV therapy must consist of the combination of two or more DAAs which inhibit different viral targets or have different mode of actions (Sarrazin and Zeuzem, 2010). Following this logic, NS5B has the great advantage of multiple inhibitor binding sites, and due to the non-overlapping resistance profiles of NS5B inhibitors targeting different binding sites, it might be possible to add more than one of them in the combination regime. It is thus still important to discover new NNIs with different binding modes and different resistance profiles even though some promising NS5B inhibitors are currently undergoing clinical investigations (Franciscus, 2011).

In this report, we describe the SPBS as novel class of HCV polymerase NNIs. Biochemical studies using the primer-dependent transcription assay, crystallisation of NS5B-inhibitor complexes, and the subgenomic HCV replicon system were used in this study