Not an open and shut helicase

By Michael J. Haas, Senior Writer

Although the serine protease domain of HCV non-structural protein 3 is one of the hottest targets in the antiviral space, little attention has been given to another enzymatic function on the same protein—a helicase that unwinds viral DNA and RNA. One reason is that the structure of HCV helicase is similar to known human proteins, thus raising concerns about off-target effects.

Now, Taiwanese researchers have identified a new chemical scaffold targeting HCV NS3 helicase. Once optimized, they said, the compounds could be less cytotoxic than previous helicase inhibitors. But companies polled by *SciBX* think that the scaffold was not sufficiently potent or nontoxic to warrant further investigation.

The group used a combination of fragment-based virtual screening and chemical synthesis to identify a triphenylmethane compound and analogs that inhibited HCV NS3 helicase activity *in vitro* and inhibited viral replication in HCV replicon cells at low micromolar concentrations.

The lead compound decreased the production of HCV RNA by 40–60% compared with that seen in untreated cells.

As reported in the *Journal of Medicinal Chemistry*, the lead compound had lower cytotoxicity in HCV replicon cells than the original triphenylmethane.¹

Group leaders were Ji-Wang Chern, professor of medicinal chemistry and dean of R&D at **National Taiwan University**, Shwu-Huey Liaw, professor of life sciences at Taiwan's **National Yang-Ming University**, and Ding-Shinn Chen, dean of National Taiwan University's College of Medicine and chairman of the hepatitis control committee of the Taiwanese government. The team also included researchers from the **Development Center for Biotechnology** and **Providence University**, both in Taiwan.

Hard target

Companies working in the HCV space said they remain skeptical about targeting HCV helicase, noting that the new scaffold did not appear to have sufficiently high specificity or sufficiently low toxicity.

Jean-Pierre Sommadossi, CEO of **Idenix Pharmaceuticals Inc.**, noted that HCV researchers have tried to target HCV NS3 helicase

for more than a decade “but have never been able to synthesize compounds with sufficient specificity to avoid interaction with cellular targets” that cause toxicity.

“I don't think this article is going to move the field very much,” he said. “Rather, it will reinforce the idea in industry that helicase is not a target where a lead with specificity can be discovered.”

Sommadosi thinks the cytotoxic effects of helicase inhibitors stem from the difficulty in targeting HCV NS3's helicase domain without also targeting its ATPase domain. ATPases are highly conserved enzymes that produce energy by converting ATP to adenosine diphosphate (ADP). Therefore, compounds that have activity against the viral protein's ATPase domain are likely to have activity against host ATPases.

Thus, Sommadossi said, “I don't know how the authors can infer less toxicity for their compounds when Table I shows there is no discrimination between the compounds' activity against helicase and ATPase.”

Team coleader Chern disagreed that the compound's inhibition of both helicase and ATPase was necessarily indicative of toxicity. “Our results showed that the lead compound inhibits NS3 helicase activity via the inhibition of both ATPase and substrate [RNA] binding—either of which is desirable to block helicase activity,” he said.

Nevertheless, in the absence of convincing data on specificity, Sommadossi didn't think the triphenylmethane scaffold was worth investigating. “You would not consider further

synthesis without a specificity 10–100” times greater for helicase than that for other targets, he said.

Moreover, he said, the lead compound's ability to inhibit the target at a 12.6 μM concentration is insufficiently potent. “We are not talking about a druggable hit unless the potency is about 1 nM or better. So these *JMC* results are off by a hundred- to a thousandfold,” Sommadossi told *SciBX*.

“There are targets that are much more amenable to HCV drugs than helicase,” he added, such as HCV NS3 protease, HCV viral polymerase and HCV non-structural protein 5A (NS5A).

Idenix's IDX184, a liver-targeting nucleotide prodrug that inhibits HCV viral polymerase, is in a Phase I/II trials. The company plans to submit INDs this year for two other compounds: IDX375, a non-nucleoside viral polymerase inhibitor, and IDX316, an HCV NS3 protease inhibitor.

Avinash Phadke, senior director of chemistry at **Achillion Pharmaceuticals Inc.**, agreed with Sommadossi about both specificity and druggability.

Phadke thought the specificity of the best compound was too low “to conclusively say that the activity seen is solely due to helicase activity.” He also noted that the active compounds showed significant cytotoxicity, whereas the nontoxic compounds were inactive, thereby

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Idenix Pharmaceuticals Inc.**

providing additional evidence for the nonselectivity of the scaffold.

Moreover, he said, “the best compound is a polyphenolic compound and I think it is not the most drug-like scaffold for further optimization.”

Achillion has two compounds in preclinical development for HCV: ACH-1095, an HCV non-structural protein 4A (NS4A) antagonist, and ACH-1625, a selective HCV NS3 protease inhibitor. The company expects to submit INDs for the compounds in 1H09 and 2H09, respectively.

Like Phadke, Malcolm MacCoss, group VP of chemical research at **Schering-Plough Corp.**’s Schering-Plough Research Institute, doubted the triphenylmethane scaffold would yield viable drug leads. “These compounds are very hydrophobic and would likely require extensive modifications of the structure to render them drug-like.” This could be challenging to accomplish while retaining the functionalities that enable helicase binding, he said.

Both MacCoss and F. George Njoroge, director of chemical research at the Schering-Plough Research Institute, noted that the modest micromolar potency of the compounds did not distinguish them from other chemical classes of helicase inhibitors.

“Intervention of the helicase activity with chemical ligands has the potential of terminating HCV proliferation,” Njoroge said. But “so far, compounds reported to inhibit HCV helicase, including antibodies, nucleoside mimics and small non-nucleoside molecules, such as *bis*-benzimidazoles and acridone derivatives, have potency in the micromolar range.”

Schering-Plough has two HCV NS3 protease inhibitors in the clinic: boceprevir is in Phase III testing and SCH900518 is in Phase II. In April, a Schering-Plough team reported the discovery of a series of second-generation NS3 inhibitors,² the best of which were 10 times more potent than boceprevir and had a similar pharmacokinetic profile, said Njoroge, who led that study.

Despite the apparent drawbacks of the triphenylmethane scaffold, Achillion’s Phadke thinks targeting HCV NS3 helicase is a worthwhile goal. “The concept that helicase inhibitors can be antiviral agents was proven *in vivo*” for herpes simple virus (HSV), he said. “I would anticipate the same in the case of HCV, if potent inhibitors are discovered.”

But, Phadke added, “whether there is any advantage in inhibiting helicase activity over protease activity is yet to be seen.”

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—Ji-Wang Chern,
National Taiwan University

At least one company is targeting a helicase to treat HSV and related herpes viruses: **Astellas Pharma Inc.**’s ASP2151, an inhibitor of the helicase-primase complex, in Phase II testing to treat genital herpes and varicella zoster virus (VZV) infection.

Building scaffold

Chern acknowledged that the lead compound of the *JMC* study was not as potent, selective

or drug-like as HCV researchers might wish. But he didn’t think those issues preclude further investigation of the triphenylmethane scaffold—which he noted was the most potent one identified by his team.

“As mentioned in our conclusion, the lead inhibitor has structural novelty which may serve as a basis for the discovery of new leads,” he said.

The team is focused on optimizing the lead to make it more drug-like while retaining the hydrophobic features of the scaffold that appear essential to its inhibitory activity, Chern said.

The findings reported in *JMC* have not been patented, but “we would like to do so when the lead optimization has been completed,” he said.

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