Proline and pyrrolidine derivatives

New drug candidates for hepatitis C treatment

Carmen Nájera and José M. Sansano

Abstract The more advantageous hepatitis C virus (HCV) inhibitors (most of them incorporating polysubstituted prolines or pyrrolidines) are detailed in this paper. The improvement of current treatments by combination of antiviral drugs is the driving force of this race to reduce the fast proliferation of this virus. The enhancement of efficiency in short periods of treatment is crucial in the economical point of view and for the hope of all infected people. New protease or polymerase inhibitors have been recently developed in order to substitute the traditional highly toxic PEG-interferon α-2b/ribavirin tandem. The contribution of our group in this field concerns the elaboration of the first and second generation GSK polymerase inhibitors through enantioselective processes based on silver(I)- and gold(I)-catalyzed 1,3-dipolar cycloadditions of azomethine ylides.

Keywords **Proline, viral inhibitor, hepatitis C virus, HCV, protease, polymerase.**

Résumé **Dérivés de proline et pyrrolidine : de nouveaux candidats médicaments pour le traitement de l'hépatite C**

Cet article décrit les inhibiteurs du virus de l'hépatite C (VHC) les plus efficaces (la plupart incorporant des prolines et pyrrolidines polysubstituées). L'amélioration des traitements actuels par une combinaison de médicaments antiviraux est le moteur de cette course pour réduire la prolifération rapide du virus. L'augmentation de l'efficacité sur de courtes périodes de traitement est cruciale du point de vue économique et pour l'espoir des personnes infectées. De nouveaux inhibiteurs de la protéase ou de la polymérase ont été récemment mis au point dans le but de remplacer le tandem traditionnel PEG-interféron α-2b/ribavirin hautement toxique. La contribution de notre groupe dans ce domaine concerne l'élaboration des première et seconde générations des inhibiteurs de polymérase GSK, grâce à des procédés énantiosélectifs basés sur des cycloadditions 1,3-dipolaires d'ylures d'azométhine catalysés par de l'argent(I) et de l'or(I).

Mots-clés **Proline, inhibiteur viral, virus de l'hépatite C, HCV, protéase, polymérase.**

Hepatitis C virus (HCV) is a blood-borne virus that has six major genotypes [1]. It enters the body through direct blood exposure and attacks cells in the liver where it replicates. HCV causes liver inflammation and kills liver cells. Up to 80% of people initially infected with HCV may become chronically infected, that is, the infection does not clear up within six months. The worst aspect of this silent virus is that most people with chronic HCV do not have symptoms and lead normal lives. However, in 10-25% of people with chronic HCV, the disease progresses over a period of 10-40 years and may lead to serious liver damage, cirrhosis (scarring) and liver cancer. Today, for example, HCV is the leading reason for liver transplants in the USA. There is currently no vaccine or cure [2], but various treatments can eradicate the virus and/or help slow or stop disease progression for some people [3].

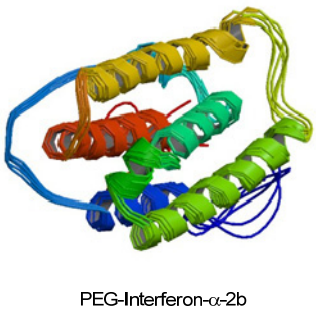
If we take in account that the two more extended global virus infections namely human immunodeficiency virus (HIV, 40 millions approximately) and HCV (more than 200 millions) are mainly transmitted by the same ways (blood to blood and vertical), the risks of co-infection become another serious problem. As many as 30% of people with HIV may also be co-infected with hepatitis C virus [4].

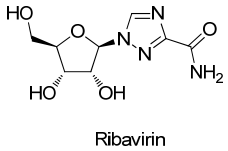
Therapies against HCV development

Recombinant pegylated interferon α-2b (PEG-IFNα-2b) [5], a HCV protease inhibitor, and ribavirin are pioneering medications for treating hepatitis C (*scheme 1*). This combined long range treatment resulted to be very expensive with many secondary effects and a low percentage of success (40-50% in genotype 1 infections).

Current research focuses on binary or ternary combinations. They are tested and evaluated in both phase II and III levels. The main component of this cocktail is PEG-IFNα-2b, which maintains a more constant level of interferon in the blood and better reduces the ability of HCV to replicate. Ribavirin, as second component, is a highly toxic oral medication used in combination with interferon to treat this infection, so alternative molecules are designed. The third component is an HCV protease or polymerase inhibitor that acts as a direct-acting antiviral agent (DAAA) inhibiting the replication process of the hepatitis C virus [6]. Ribavirin and HCV DAAA, for example, are not effective when used alone and frequently require the presence of PEG-IFNα-2b.

These DAAAs target specific key steps of the viral life cycle. They inhibit the HCV protease or the HCV polymerase.





Scheme 1 - PEG-interferon-α-2b and ribavirin, two key components of the therapy against hepatitis C virus.

Unfortunately, these agents are no longer effective by themselves due to the high levels of resistance caused by the fast HCV mutations.

At this moment, people infected by HCV genotype 1 who take the triple combination have up to a 91% chance of curing hepatitis C according to some particular phase III trials. Treatment can be tailored to 24, 36 or 48 weeks total treatment duration. People with HCV genotype 2 or 3 are typically treated with a combination of pegylated interferon plus ribavirin (without an HCV protease inhibitor). In this case, the chances of being cured are up to 82%. Total treatment duration is usually 24 weeks. An alternative to the remaining 18% of non-cured infected people is based on a triple combination as described above.

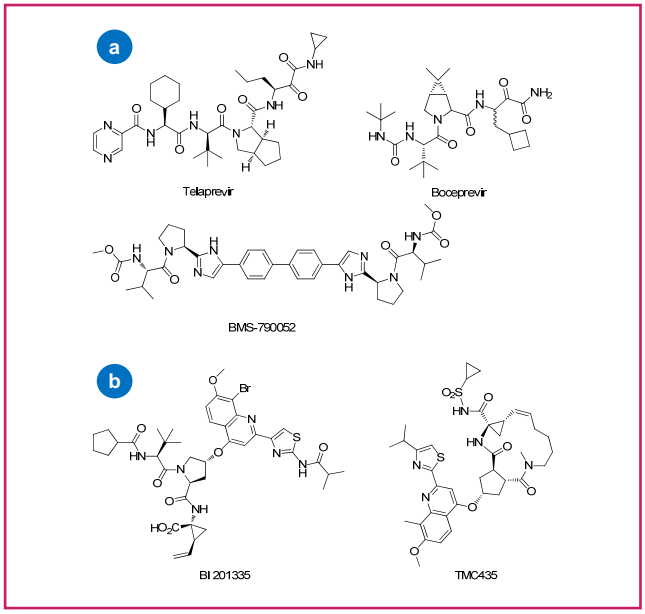
The most common side effects of interferon, ribavirin and an HCV protease inhibitor include mild flu-like symptoms, muscle and joint pain, nausea, headaches, fatigue, loss of appetite, dry skin, anxiety, depression and insomnia. For some people, physical side effects are worse when the drug is started and may diminish over time. The most common reason for stopping HCV therapy is anemia (low red blood cells count), thrombocytopenia (low platelet count), and neutropenia (low white blood cells count).

New DAAA drugs [7]

These new medications supplement – not replace – the traditional treatment by blocking specific sites of the corresponding viral proteins according to known structure-activity relationships (SAR). Phase IV (commercially available) and phase III protease inhibitors (*scheme 2* and *table*) incorporate a proline or a pyrrolidine nucleus except cyclopeptide DEBio025. Proline or proline derivatives are very important natural and synthetic compounds with very interesting chemical and bioactive applications. They are mainly useful in SAR studies of themselves or proteins/synthetic molecules containing them [8-9], in the synthesis of pyrrolidine core-based natural products and other applications in many scientific fields.

The triple combination of PEG-IFNα-2b/ribavirin/telaprevir is the most efficient and shorter approved treatment (requires 12 weeks) unlike to other combinations incorporating boceprevir (28-48 weeks) or BMS-790052 (24 weeks).

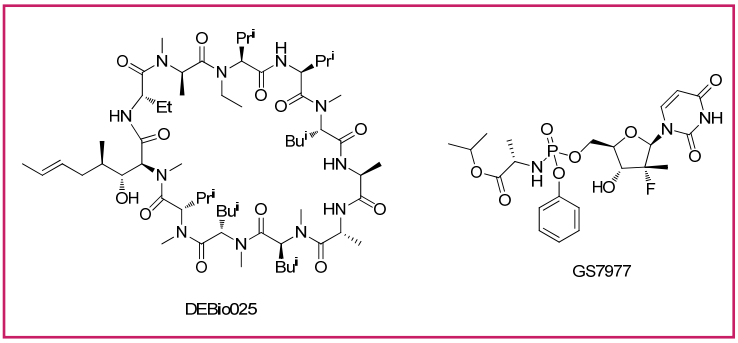
Polymerase inhibitors are not as developed as protease ones such as it is demonstrated by the latest results obtained in several clinical stages. Most promising polymerase blockers DEBio025 (indirect blocker) and GS7977 are going to reach the market very soon (*scheme 3*). Whilst DEBio025 is an immunosuppresor acting on cyclophilin-B, a binding



Scheme 2 - a) Phase IV (commercially available) protease inhibitors, and b) phase III protease inhibitors.

Table - Name and status of the most promising DAAA drugs acting as inhibitors of hepatitis C virus.

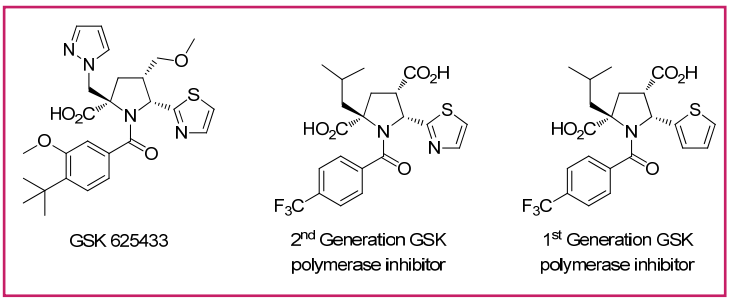
Protease inhibitors	Phase	Polymerase inhibitors	Phase
Telaprevir	IV	DEBio025	III
Boceprevir	IV	GS7977	III
BI 201335	III		
BMS-790052	III		
TMC435	III		



Scheme 3 - Phase III polymerase inhibitors.

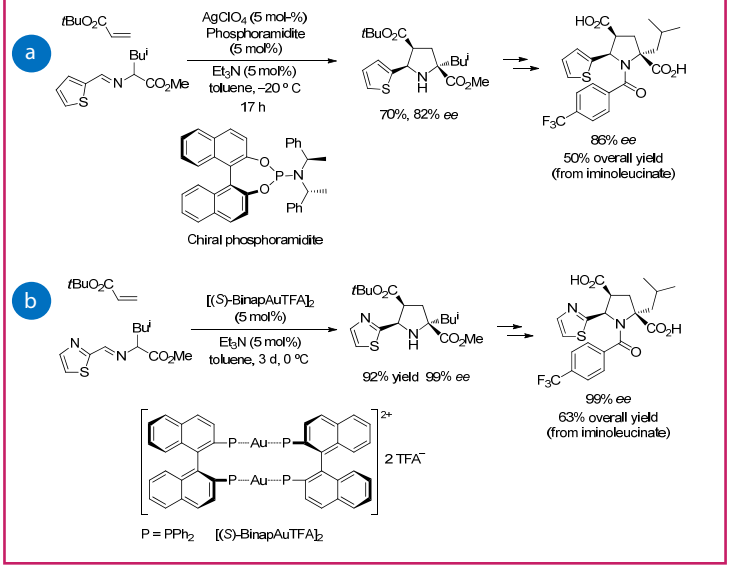
protein that plays a role in hepatitis C virus (HCV) replication, Gilead made a big wager on the uracil nucleotide analogue GS7977 because it has been considered as the first all oral treatment for the hepatitis C [10]. But at this moment, GS7977 with PEG-IFNα-2b/ribavirin cures most treatment-naive hepatitis C patients in 12 weeks.

There are dozens of compounds in the pipeline waiting for FDA approval but many of them failed in whatever part of this long and difficult way of drugs. For example, the very promising *in vivo* results of proline-core antiviral agent GSK 625433 terminated earlier after phase I trials (November 2011) (*scheme 4*) [6].



Scheme 4 - GSK polymerase inhibitors.

We developed in our group the asymmetric synthesis of the first and second generation antiviral drugs by 1,3-dipolar cycloaddition (as key-step) between the corresponding methyl iminoleucinate and *tert*-butyl acrylate employing a chiral phosphoramidite-$AgClO_4$ [11] (*scheme 5a*) and a chiral dimeric binap-gold(I) complex [12] (*scheme 5b*), respectively. In both routes, the overall yields obtained were moderate to good and enantioselectivities very high, especially in the case of the second generation inhibitor (99% *ee*). In this moment, the synthetic approach of the third generation GSK 625433 polymerase inhibitor, much more active than the previous first and second ones, is currently underway.



Scheme 5 - Asymmetric synthesis of the first and second generation GSK polymerase inhibitors.

Now, risks of hepatitis C became more serious when appear as co-infection in people with HIV. It is estimated that approximately 1.2 million people in the USA are infected with HIV and more than 4 million are infected with hepatitis C as well. The different treatments (36-44-50 weeks) in phases III-IV are based on the possible multiple combination of PEG-IFNα-2b/ribavirin, and one of the DAAA drugs depicted in the *table*.

As summary, the hope of cure of infected patients is getting higher and higher and new effective drugs with more reduced side effects are envisaged. Nucleotide derived agents are demonstrated to be more resistant to mutations of this virus responsible of the hepatitis C. The search for the ideal single drug (not combined with other antivirals), whose smaller dose is able to reduce completely the infection with negligible side effects, is the main objective of scientists.

Acknowledgements

This work has been supported by the DGES of the Spanish Ministerio de Ciencia e Innovación (MICINN) (Consolider INGENIO 2010 CSD2007-00006, CTQ2007-62771/BQU, CTQ2007/67528, CTQ2010-20387), FEDER, the Hispano-Brazilian project PHB2008-0037-PC, the Generalitat Valenciana (PROMETEO/2009/039), the Basque government (Grant IT-324-07), and the University of Alicante. The authors also thank the SGI/IZO-SGIker of UPV/EHU for allocation of computational resources.

Notes and references

[1] Brody H., Hepatitis C, *Nature*, **2011**, *474*, p. S1.
[2] An investigational hepatitis C virus (HCV) therapeutic vaccine significantly improved the likelihood of sustained response to interferon-based therapy, according to a proof-of-concept study reported at the 47th International Liver Congress (EASL April 30th 2012).
[3] www.hcvadvocate.org/hepatitis/hepatitis_C.asp
[4] www.hcvadvocate.org/hepatitis/factsheets_pdf/Coinfection%20Guide.pdf
[5] Although other interferons are being evaluated, this is the most efficient drug for current treatments.
[6] Mayhoub A.S., Hepatitis C RNA-dependent RNA polymerase inhibitors: A review of structure-activity and resistance relationships; different scaffolds and mutations, *Bioorg. Med. Chem.*, **2012**, *20(10)*, p. 3150.
[7] www.hcvadvocate.org/hepatitis/hepC/HCVDrugs_2012.pdf
[8] a) Calaza M.I., Cativiela C., Stereoselective synthesis of quaternary proline analogues, *Eur. J. Org. Chem.*, **2008**, p. 3427 ; b) Companyó X., Alba A.N., Rios R., Most relevant recent enantioselective synthesis of pyrrolidines and piperidines, O.A. Attanasi, D. Spinelli (eds), *Targets in Heterocyclic Systems – Chemistry and properties*, *vol. 13*, **2009**, RSC, Cambridge, p. 147.
[9] Zhang J., Pan X., Wang C., Wang F., Li P., Xu W., He L., Pharmacophore modeling, 3D-QSAR studies, and *in-silico* ADME prediction of pyrrolidine derivatives as neuraminidase inhibitors, *Chem. Biol. Drug. Des.*, 2012, *79*, p. 353.
[10] McCoy M., Gilead places a huge bet, *Chem. Eng. News*, **2011**, *89(48)*, p. 8.
[11] Nájera C., Retamosa M.G., Martín-Rodríguez M., Sansano J.M., de Cózar A., Cossío F.P., Synthesis of prolines by enantioselective 1,3-dipolar cycloaddition of azomethine ylides and alkenes catalyzed by chiral phosphoramidite-silver(I) complexes, *Eur. J. Org. Chem.*, **2009**, *32*, p. 5622.
[12] Martín-Rodríguez M., Nájera C., Sansano J.M., de Cózar A., Cossío F.P., Chiral gold(I) vs chiral silver complexes as catalysts for the enantioselective synthesis of the second generation GSK-hepatitis C virus inhibitor, *Beilstein J. Org. Chem.*, **2011**, *7*, p. 988.

Carmen Nájera et **José M. Sansano** sont professeurs à l'Université d'Alicante (Espagne)*.
***Carmen Nájera a reçu le Prix franco-espagnol « Miguel Catalán-Paul Sabatier » 2010 de la Société Chimique de France**.

C. Nájera **J.M. Sansano**

* Instituto de Sintesis Organica, Departamento de Química Orgánica, Universidad de Alicante, Apdo. 99, E-03080-Alicante.
Courriels : cnajera@ua.es, jmsansano@ua.es
** www.societechimiquedefrance.fr/fr/prix-franco-espagnol.html