Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 14 (2004) 5333–5337

HCV NS5B polymerase-bound conformation of a soluble sulfonamide inhibitor by 2D transferred NOESY

Constantin G. Yannopoulos,[a,*] Ping Xu,[b] Feng Ni,[b] Laval Chan,[a] Oswy Z. Pereira,[c] T. Jagadeeswar Reddy,[c] Sanjoy K. Das,[a] Carl Poisson,[a] Nghe Nguyen-Ba,[a] Nathalie Turcotte,[a] Melanie Proulx,[c] Lilianne Halab,[a] Wuyi Wang,[c] Jean Bédard,[a] Nicolas Morin,[c] Martine Hamel,[c] Olivier Nicolas,[a] Darius Bilimoria,[a] Lucille L'Heureux,[a] Richard Bethell[c] and Gervais Dionne[a]

[a]*ViroChem Pharma Inc., 275 Armand-Frappier Blvd., Laval, QC, Canada H7V 4A7*
[b]*Biotechnology Research Institute, 6100 Royalmount Avenue, Montreal, QC, Canada H4P 2R2*
[c]*Shire BioChem Inc., 275 Armand-Frappier Blvd., Laval, QC, Canada H7V 4A7*

Received 14 June 2004; revised 9 August 2004; accepted 9 August 2004
Available online 8 September 2004

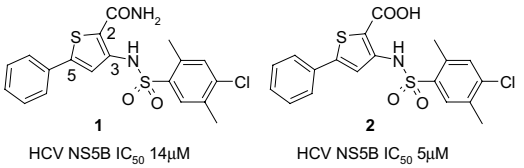
Abstract—HCV NS5B RNA-dependent RNA polymerase (NS5B) is essential for viral replication and is therefore considered a target for antiviral drug development. From our ongoing screening effort in the search for new anti-HCV agents, a novel inhibitor **1** with low μM activity against the HCV NS5B polymerase was identified. SAR analysis indicated the optimal substitution pattern required for activity, for example, carboxylic acid group at 2-position of thiophene ring. We describe the steps taken to identify and solve the bioactive conformation of derivative **6** through the use of the transferred NOE method (trNOE).

Transferred NOE[1] (trNOE) is increasingly used in the drug discovery[2] process, as it provides structural insights about the conformation of an inhibitor bound to an appropriate macromolecular target. In order to observe trNOEs, the inhibitor has to undergo rapid on/off exchange between the free and target-bound state making this method particularly attractive during the early steps of a drug discovery program. We have successfully applied the trNOE method and provided structural insight to our ongoing development of antiviral agents against the Hepatitis C virus (HCV). The urgent need of new anti-HCV agents is accentuated by increasing global infection levels to more that 170 million and the lack of effective therapies or vaccines. In addition, the incidence increases by 3–4 million per year,[3] and it is estimated that 80% of those infected will develop chronic infection, and about 20% and 5% will develop cirrhosis and hepatocellular carcinoma, respectively.[4,5]

HCV is a member of the Flaviviridae family and is a single-stranded positive RNA[6] virus that encodes a large polypeptide (9.6 kb; about 3000 amino acids). Complete polypeptide maturation yields structural C, E1, E2 and non-structural NS2, NS3, NS4A, NS4B, NS5A, NS5B proteins. It has been demonstrated that the virally encoded NS5B RNA-dependent RNA polymerase is essential for viral replication[7–9] and is therefore considered a viable target for antiviral drug development. Three groups have solved independently the NS5B crystal structure[10–12] and no bioactive solution conformation has been reported for any of the known inhibitors.[13] Recently, we have reported the first X-ray structure of a N,N-disubstituted phenylalanine bound to NS5B polymerase at an allosteric site[14] followed by structure–activity relationship (SAR) studies.[15a,b] Our ongoing efforts in the search for new anti-HCV agents resulted in the discovery of a novel inhibitor **1** with low μM activity against HCV polymerase. SAR analysis indicated that the carboxylic acid at position 2 of the thiophene was beneficial for activity; carboxylic acid **2** was almost threefold more potent than the corresponding carboxamide **1**. The complete details of our optimization program are described elsewhere.[15c,d]

Keywords: HCV; NS5B Polymerase; NMR; Inhibitor.
* Corresponding author. Tel.: +1 450 978 6435; fax: +1 450 978 7777; e-mail: cyannopoulos@virochempharma.com

0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.08.018



In this report, we describe the steps taken to determine the bioactive conformation of sulfonamide derivative **6** through the use of trNOE. Weakly binding sulfonamide **2** was chosen as a starting point for screening by 1D proton NMR differential line broadening (DLB) as a measure of specific complexation. Although **2** exhibited DLB upon addition of the HCV NS5B polymerase pre-complexed with poly[rA]/oligo[dT] template-primer,[16] no trNOEs were observed. Low concentration of the bound state probably resulted in undetectable trNOEs due to the observed low solubility of the inhibitor-polymerase complex. It is interesting to note that in the absence of the template-primer, weak DLB was observed and no DLB resulted from template-primer in absence of NS5B. Therefore, the presence of template-primer appears to play an important role in the integrity of the binding site.

Synthetic efforts (Schemes 1 and 2) were therefore undertaken to increase the solubility of this class of inhibitors by introducing a pyridyl ring on either the 5- or 3-positions of the thiophene ring. Introducing a 2-pyridyl function at the 3-position of the thiophene ring (Compound **3**) retained weak anti-NS5B activity (IC_{50} 4 μM) and increased solubility by 10-fold. Unfortunately no trNOEs were observed due to extensive protein precipitation within 2 h after complex formation.

We therefore explored the possibility of introducing a 4-pyridyl function at the 5-position of the thiophene ring and pyridine analog **5** was found to retain weak anti-NS5B activity (IC_{50} 4 μM). The solubility of **5** was similar to that of **3** but the complex stability was still less than 2 h. Weaker binders such as **4** (IC_{50} 15 μM) and **6** (IC_{50} 25 μM) were then evaluated. DLB was observed for both analogues and surprisingly the weakest pyridyl analogue **6** exhibited the best NS5B induced DLB. In addition, the ternary complex was stable for almost 4 h and therefore **6** was chosen for NMR data collection.

Key trNOE correlations between the pyridyl ring proton [**a**] (see labeled structure in Fig. 1), phenyl protons [**f**,**g**,**h**], and the thiophene proton [**e**] can be clearly seen in the 2D water flip-back ^{1}H NOESY spectrum of **6**

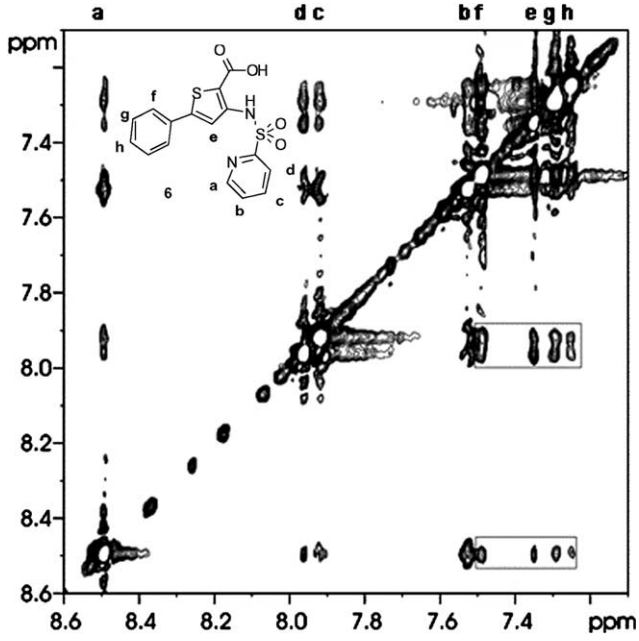
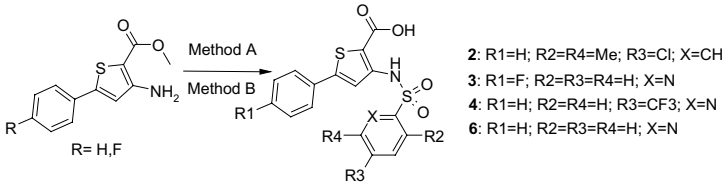
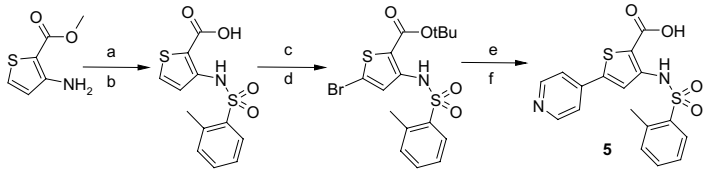


Figure 1. The water flip-back 2D-NOESY for bound **6** at 800 MHz and 298 K, with a mixing time of 50 ms. The boxed NOE cross-peaks appear upon NS5B binding and are of same sign as the diagonal peaks.



Scheme 1.



Scheme 2. Reagents and conditions: Method A (i) LiOH, dioxane, H_2O, reflux; (ii) $ArSO_2Cl$, Na_2CO_3, dioxane, H_2O. Method B (i) $ArSO_2Cl$, DMAP, CH_2Cl_2, H_2O; (ii) LiOH, dioxane, H_2O, reflux. (a) *o*-Toluenesulfonyl chloride, Et_2O, DMAP, CH_2Cl_2. (b) LiOH, dioxane. (c) 2-Propene, H_2SO_4, dioxane, 60 °C. (d) LDA, $(BrCF_2)_2$, −78 °C. (e) 4-Pyridyl-$B(OH)_2$, $Pd(PPh_3)_4$. (f) TFA, CH_2Cl_2, rt.

(boxed cross-peaks) shown in Figure 1. Pyridyl ring proton [**d**] also correlated with phenyl [**f**,**g**,**h**] and thiophene [**e**] protons, but to a weaker extent as compared to proton [**a**] correlations. This observation was key in deducing the orientation of the pyridyl nitrogen with respect to proton [**a**] and thereof putting the [**a**] proton closest to the thiophene proton [**e**]. Pyridyl proton [**c**] correlated equally as [**a**] with all phenyl and thiophene protons.

The observed trNOE correlations maximized when recorded at mixing times between 50 and 70 ms and no NOE's were observed for the free sulfonamide **6**. Conformational searches[17] generated low energy structures (Fig. 2A) that could not explain the observed trNOE correlations. Applying the strongest NOE contacts [**d**–**f**] and [**a**–**e**] at ~4 Å range followed by [**a**–**f**] at 4–5 Å range quickly shaped **6** into a 'bent' structure that was

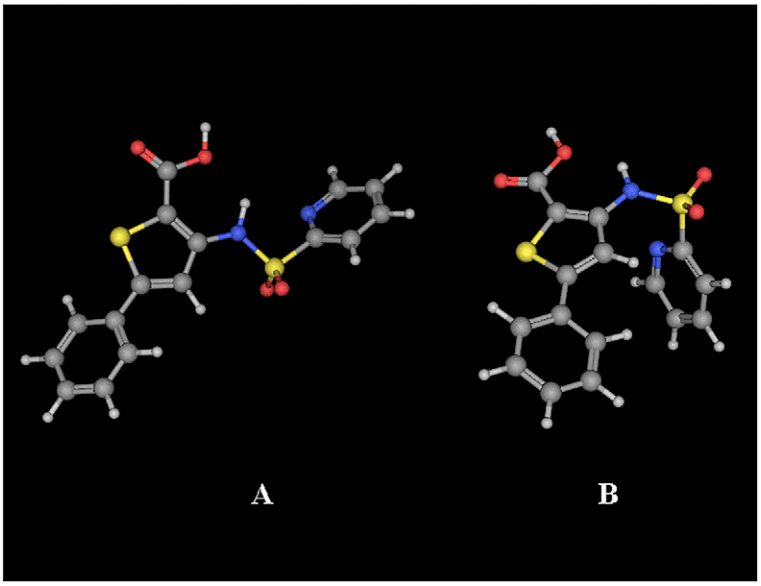


Figure 2. (A) Lowest energy structure obtained from stochastic conformational search. (B) Low energy structure derived from NMR data of **6** when bound to NS5B.

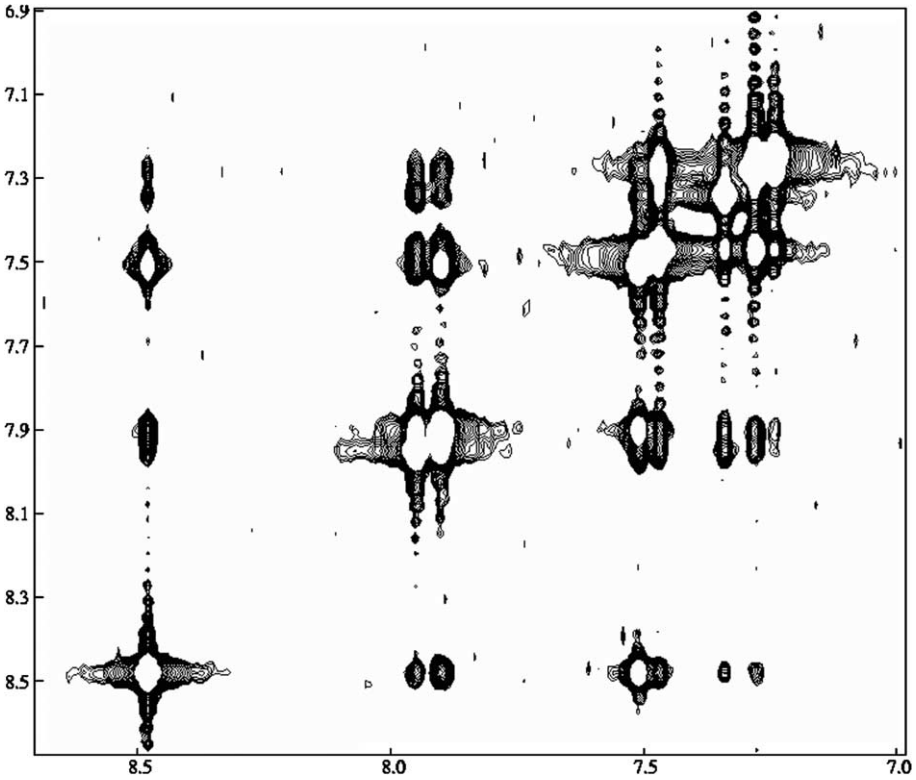


Figure 3. Back-calculated simulated NOESY of the NMR-derived bent-shape sulfonamide **6**.

not very different to the final NMR derived structure as depicted in Figure 2B. The trNOE back-calculations of this bent shape of **6** reproduced all intense experimental trNOE's (more than 98% of boxed cross-peaks seen in Fig. 1) as shown in Figure 3.[18]

The very weak trNOE's were not considered in the structure calculations or in the back-calculations as they may arise from complex-mediated spin diffusion which is yet of unknown nature.

In summary, a trNOE structure of a soluble sulfonamide is reported; the 'bent' shape character provided the first understanding towards better inhibitor design. The solution conformation of **6** bound to HCV NS5B polymerase has also been confirmed by X-ray crystallography of a related sulfonamide analogue/NS5B complex and these findings will be described elsewhere. Finally, we are attempting to understand the role of the template-primer since this study indicates that adequate inhibitor binding to the NS5B is dependent on the presence of template-primer.

Acknowledgements

We thank Thérèse Godbout for her assistance in the preparation of this manuscript.

References and notes

1. (a) Clore, G. M.; Gronenborn, A. M. *J. Magn. Reson.* **1982**, *48*, 402; (b) Ni, F.; Scheraga, H. A. *Acc. Chem. Res.* **1994**, *27*, 257.
2. (a) Gonnella, N. C.; Bohacek, R.; Zhang, X.; Kolossvary, I.; Paris, C. G.; Melton, R.; Winter, C.; Hu, S.-I.; Ganu, V. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*(2), 462; (b) LaPlante, S. R.; Aubry, N.; Bonneau, P. R.; Kukolj, G.; Lamarre, D.; Lefebvre, S.; Li, H.; Llinas-Brunet, M.; Plouffe, C.; Cameron, D. R. *Bioorg. Med. Chem. Lett.* **2000**, *10*(20), 2271.
3. *Hepatitis C: Global Prevalence*. Weekly Epidemiological Record, **2000**; Vol. 75, p 3.
4. WHO **1999**, *J. Viral Hepatitis 6*, 35.
5. Saito, I.; Miyamura, T.; Ohbayashi, A.; Harada, H.; Katayama, T.; Kikuchi, S.; Watanabe, Y.; Koi, S.; Onji, M., et al. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 6547.
6. Purcell, R. *Hepatology* **1997**, *26*, 11S.
7. Takamizawa, A.; Mori, C.; Fuke, I.; Manabe, S.; Murakami, S.; Fujita, J.; Onishi, E.; Andoh, T.; Yoshida, I.; Okayama, H. *J. Virol.* **1991**, *65*, 1105.
8. Kato, N.; Hijikata, M.; Ootsuyama, Y.; Nakagawa, M.; Ohkoshi, S.; Sugimura, T.; Shimotohno, K. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9524.
9. Grakoui, A.; Wychowski, C.; Lin, C.; Feinstone, S. M.; Rice, C. M. *J. Virol.* **1993**, *67*, 1385.
10. Ago, H.; Adachi, T.; Yoshida, A.; Yamamoto, M.; Habuka, N.; Yatsunami, K.; Miyano, M. *Structure* (*London*) **1999**, *7*, 1417.
11. Lesburg, C. A.; Cable, M. B.; Ferrari, E.; Hong, Z.; Mannarino, A. F.; Weber, P. C. *Nat. Struct. Biol.* **1999**, *6*, 937.
12. Bressanelli, S.; Tomei, L.; Roussel, A.; Incitti, I.; Vitale, R. L.; Mathieu, M.; De Francesco, R.; Rey, F. A. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 13034.
13. For a recent review on HCV NS5B polymerase patent literature, see: Zhang, X. *Idrugs* **2002**, *5*, 154.
14. Wang, M.; Ng, K. N. S.; Cherney, M. M.; Chan, L.; Yannopoulos, C. G.; Bédard, J.; Morin, N.; Nguyen-Ba, N.; Bethell, R. C.; Alaoui-Ismaili, M. H.; James, M. N. G. *J. Biol. Chem.* **2003**, *278*, 9489.
15. (a) Chan, L.; Reddy, T. J.; Proulx, M.; Das, S. K.; Pereira, O.; Wang, W.; Siddiqui, A.; Yannopoulos, C. G.; Poisson, C.; Turcotte, N.; Drouin, A.; Alaoui, H. M. A.; Bethell, R.; Hamel, M.; Bilimoria, D.; L'Heureux, L. C. *J. Med. Chem.* **2003**, *46*, 1283; (b) Reddy, T. J.; Chan, L.; Turcotte, N.; Proulx, M.; Pereira, O. Z.; Das, S. K.; Siddiqui, A.; Wang, W.; Poisson, C.; Yannopoulos, C. G.; Bilimoria, D.; L'Heureux, L.; Alaoui, H. M. A.; Nguyen-Ba, N. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3341; (c) Chan, L.; Reddy, S. K.; Das, S. K.; Reddy, T. J.; Poisson, C.; Proulx, M.; Pereira, O.; Courchensne, M.; Roy, C.; Wang, W.; Siddiqui, A.; Yannopoulos, C. G.; Nguyen-Ba, N.; Labrecque, D.; Bethell, R.; Hamel, M.; Courtemanche-Asselin, P.; L'Heureux, L.; David, M.; Nicolas, O.; Brunette, S.; Bilimoria, D.; Bedard, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 793; (d) Chan, L.; Pereira, O.; Reddy, T. J.; Das, S. K.; Poisson, C.; Courchensne, M.; Proulx, M.; Siddiqui, A.; Yannopoulos, C. G.; Nguyen-Ba; Roy, C.; Nasturica, D.; Moinet, C.; Bethell, R.; Hamel, M.; L'Heureux, L.; David, M.; Nicolas, O.; Courtemanche-Asselin, P.; Brunette, S.; Bilimoria, D.; Bedard, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 797.
16. The expression and purification of HCV NS5B genotype 1b strain BK is described in our publication (see Ref. 15a).
17. Sulfonamide **6** was studied by modeling the structure at the ionization state that could be reflected in aqueous conditions at ~pH 7. MOE (Chemical Computing Group Inc., Montreal, Canada) was used to build and energy minimize **6** with the MMFF94s force field using a gradient of 0.01 kcal/mol Å. The resulting minimized structure was used as a starting point for generating a set of conformers obtained from the use of MOE's implemented systematic conformational searching tool. To visualize the conformational preference of **6**, the first 50 lowest energy structures were examined for each of the representative 'closed' or 'open' shapes. Only the 'open' shape of **6** was observed. The average energy difference between the 1st and 50th structure was approximately 2–3 kcal/mol Å.
18. The MOE minimized structure of **6** was used as starting points for introducing distance restrains observed from the trNOE spectrum. The strongest observed H–H correlations were initially assigned to distances of 4–5 Å and imposed geometric constraints were set with strengths of 100. Conformations of **6** consistent with observed NOEs were generated by minimization using the MMFF94s force field and were saved as .pdb files needed for trNOE back-calculations using the trNOEPAK program. trNOEPAK is part of the ECEPP/NMR (ECEPP algorithm was originally developed in Prof. Scheraga's group at Cornell University) suite that was developed by Dr. Feng Ni group at the Biotechnology Research Institute and is currently used for conformational structure calculations/validations through comparisons of experimental and calculated transferred NOE18 spectra. The conformer .pdb file was used by the PDB2NOE program (part of ECEPP/NMR) in order to calculate the predicted transferred NOE intensities that were saved into a .noe file. The .noe was used by GFIDSJ (part of ECEPP/NMR) to generate a free-induction decay (FID) matrix (.dat file) using the calculated NOEs and the experimental chemical shifts, line widths, coupling constants and attenuation factors for all observable protons. Finally, the NMRDSP program (part of ECEPP/NMR) was used as a digital signal processing

system for converting the simulated and corresponding experimental FID matrices into the NOESY spectrum .mat file. NMRVIEW 5.0.2 was used to visualize the simulated .mat spectrum seen in Figure 3. Additional

references: Nemethy, G.; Gibson, K. D.; Palmer, K. A.; Yoon, C. N.; Paterlini, G.; Zagari, A.; Rumsey, S.; Scheraga, H. A. *J. Phys. Chem.* **1992**, *96*, 6472; (b) Ni, F. *Prog. NMR Spectrosc.* **1994**, *26*, 517.