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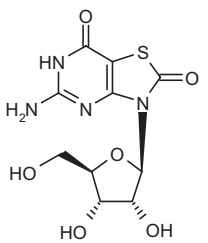
Isatoribine

Prop INN; USAN

Anti-Hepatitis C Virus Drug
TLR7 Receptor Agonist

Satoribine (former USAN)
ANA-245

7-Thia-8-oxoguanosine
5-Amino-3-(β-D-ribofuranosyl)thiazolo[4,5-*d*]pyrimidine-2,7(3*H*,6*H*)-dione



$C_{10}H_{12}N_4O_6S$
Mol wt: 316.2916

CAS: 122970-40-5

CAS: 198832-38-1 (as monohydrate)

EN: 149064

Abstract

Current therapeutic intervention for hepatitis C virus (HCV) infection is unsatisfactory, and due to the virus's high genetic heterogeneity, it is important that new therapies target a broad range of viral genotypes. A novel immunomodulator, isatoribine, is currently undergoing phase Ib development for HCV. This toll-like receptor 7 (TLR7) agonist has demonstrated potent immunogenicity *in vitro* and *in vivo*, providing significant protection against a broad spectrum of viral challenges. Interim clinical data in patients with chronic HCV have indicated that daily i.v. doses of 800 mg significantly reduce plasma HCV RNA levels, modulate interferon-responsive genes and are effective against genotypes 1 and 3. The outcome of further studies using oral prodrugs of isatoribine, namely ANA-975 and ANA-971, will also be discussed. These novel candidates for HCV represent promising new therapeutic strategies.

Synthesis

Reaction of dimethyl 2-bromomalonate (I) with 2-(trimethylsilyl)ethanethiol (II) by means of K_2CO_3 in THF gives dimethyl 2-(2-trimethylsilylethylsulfanyl)malonate (III), which is cyclized with guanidine (IV) by means of MeONa in MeOH to yield the pyrimidinone (V). Halogenation of compound (V) with $POCl_3$ and pyridine affords the dichloropyrimidine (VI), which is condensed with the glycoside (VII) in refluxing ethanol to provide the adduct (VIII). Acylation of adduct (VIII) with ethyl chloroformate (IX) and NaOH gives the carbamate (X), which is cyclized by means of TBAF in THF to yield the thiazolopyrimidine (XI). Treatment of compound (XI) with MeONa affords the corresponding methoxy derivative (XII), which is treated with trimethylsilyl iodide to provide the bicyclic dione (XIII). Finally, the acetonide group of (XIII) is hydrolyzed in acid medium (1). Scheme 1.

Introduction

Hepatitis C virus (HCV) is an enveloped RNA virus belonging to the Flaviviridae family. It is primarily contracted via direct percutaneous exposure to infected blood, usually during intravenous drug use, but transmission can also commonly occur via transfusion and hemodialysis. Infrequent exposure has been reported via perinatal exposure (2), needle-stick injuries in healthcare workers, tattooing and sexual contact (3) (see Fig. 1).

HCV infection initially causes acute hepatitis, with 3-4 million people newly infected with HCV each year according to World Health Organization (WHO) estimates. Symptoms of HCV include jaundice, fatigue, abdominal

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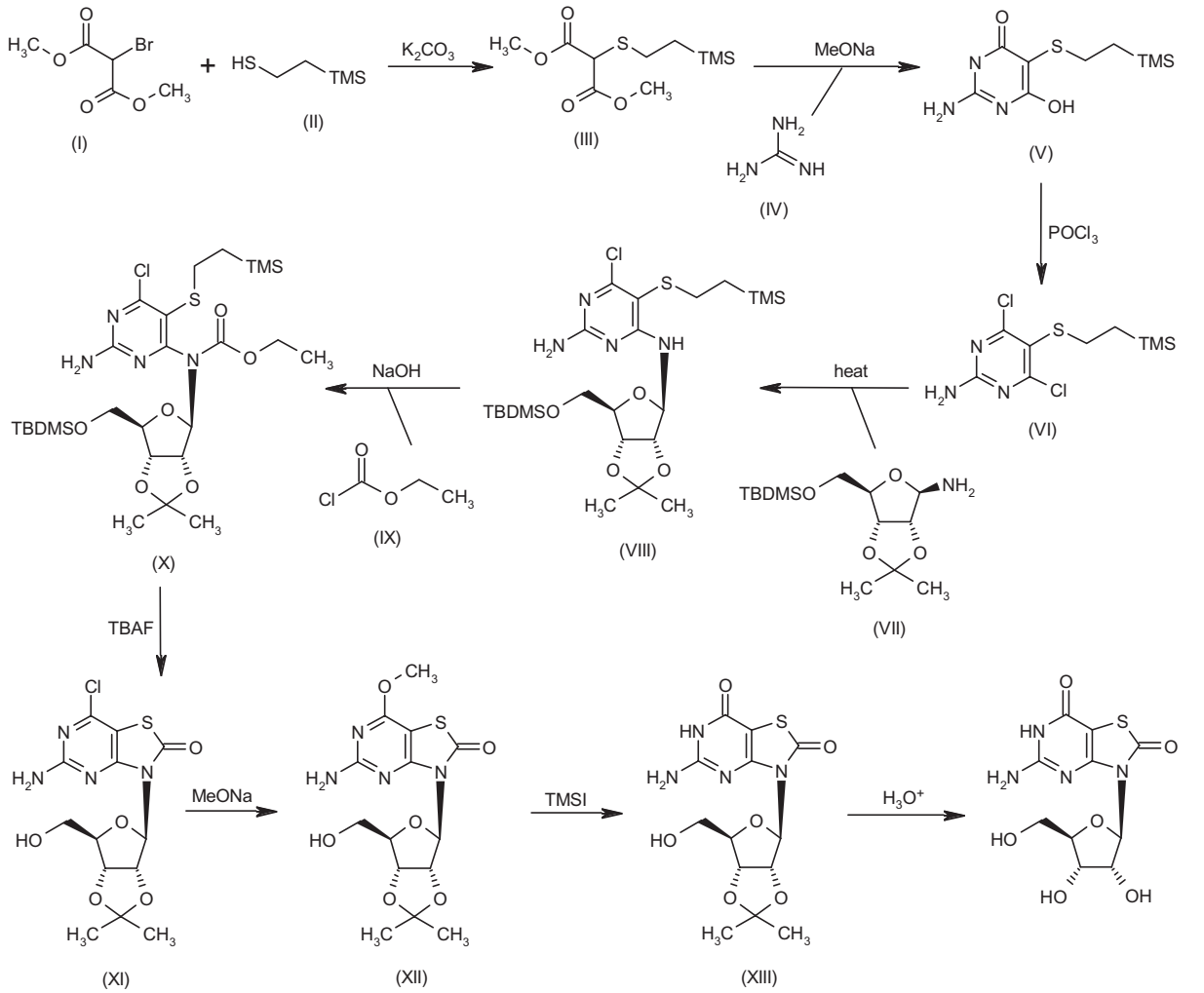
Scheme 1: Synthesis of Isatoribine



pain, loss of appetite and intermittent nausea and vomiting; however, it is not uncommon for those infected to remain symptom-free until the chronic stage, potentially infecting others (4). Approximately 70% of individuals with acute HCV infection progress to chronic HCV in the U.S. (5), while the WHO estimates that 3% of the world's population has chronic HCV (3). These numbers may, however, be greatly underestimated as high-risk groups such as the institutionalized or homeless, who are often at the greatest risk, are frequently not accounted for (6). Chronic and progressive HCV is associated with significant morbidity and mortality and is a major cause of cirrhosis, end-stage liver disease and liver cancer (7). Existing cases of HCV represent a significant healthcare and economic burden, and the Centers for Disease Control (CDC) estimates that medical and work-related costs incurred by HCV in the U.S. exceed USD 600 million (3).

Current therapy for HCV is limited to a combination of pegylated interferon and ribavirin, but this strategy is effective in less than 50% of cases of HCV genotype 1, the most predominant genotype in Western countries. Therefore, improved and/or novel treatments are needed and several different strategies are being pursued, including immunomodulating agents (8-11).

Isatoribine (ANA-245) is a toll-like receptor 7 (TLR7) agonist originally identified at the former ICN as a promising immunotherapeutic from a series of guanosine analogues and derivatives (12). TLR7 belongs to a family of pathogen recognition receptors which initiate the innate immune response via the release of cytokines and the activation of pathways and enzymes that destroy intracellular pathogens, while at the same time activating immature dendritic cells to prepare for their differentiation into antigen-presenting cells (APCs) (13, 14). Isatoribine has completed phase Ia studies. As a result of the success-

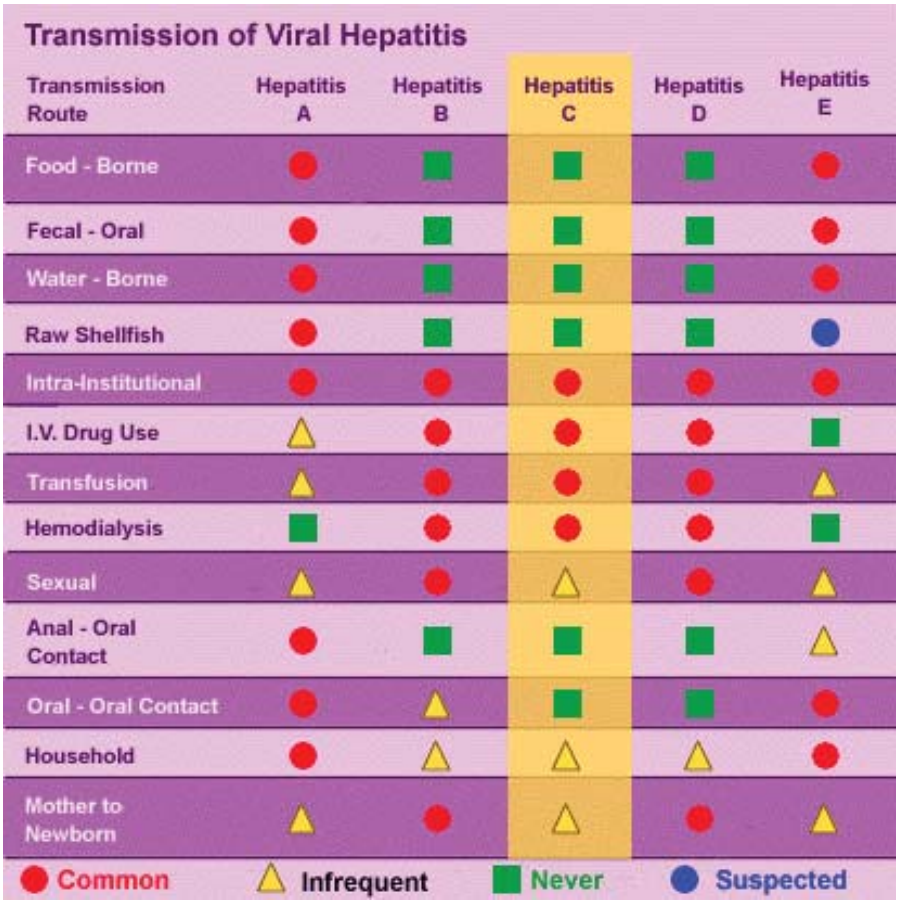


Fig. 1. Transmission of viral hepatitis (from Prous Science Integrity®; reproduced with permission from www.epidemic.org).

ful development of isatoribine, Anadys has produced two oral prodrugs of this compound (ANA-975 and ANA-971) as potential therapies for HCV (15).

Pharmacological Actions

Primary *in vitro* analysis of a number of thiazolo[4,5-*d*]pyrimidine nucleosides for immunomodulating activity revealed that isatoribine demonstrated the most effective induction of *de novo* DNA synthesis in murine spleen cells, with concentration-dependent and noncytotoxic mitogenic activity and a potent 50-fold increase in thymidine incorporation at 0.4 mM. This analogue also augmented murine natural killer (NK) cell activity against T-cell lymphoma target cells (YAC-1) in an *ex vivo* system, demonstrating the highest activity with regard to effector:target ratios (cytotoxicity = 74.8% and 67.8%, respectively, at effector:target ratios of 50:1 and 100:1) (12).

The effect of isatoribine on the proliferation of thymocytes was studied *in vitro*. The compound was found to stimulate the proliferation of thymocytes in a concentration- and time-dependent manner, with maximum effects at 500 μM at 3-4 days. Proliferation was also dependent on the presence of APCs. Isatoribine stimulated IL-2 production by thymocytes and the expression of the activation markers CD25 and CD71. An upregulation of CD25 (α subunit of the IL-2 receptor, IL-2Rα) was detected on both thymocytes and thymic dendritic cells. The application of a mixture of two anti-CD25 monoclonal antibodies inhibited isatoribine-stimulated cell proliferation by 65-85%, confirming that its effect is mediated mainly by IL-2 (16).

Further studies confirmed the involvement of IL-2 in isatoribine's immunomodulatory effects. Isatoribine significantly increased the proliferation of rat thymocytes stimulated by suboptimal concentrations of concanavalin A, with maximal effect seen at 250 μM. These effects correlated with increased IL-2 production and upregulation of IL-2Rα expression and decreased apoptosis of thymocytes (17, 18).

In vitro in human peripheral blood mononuclear cells (hPBMCs), isatoribine concentration-dependently stimulated the production of interferon alfa, beta and gamma, IP-10 (interferon gamma-inducible protein-10), ISG15 (an interferon-stimulated ubiquitin-like protein) and 2'-5'-

oligoadenylate synthetase (OAS). These isatoribine-activated hPBMCs potently inhibited HCV replication in the subgenomic replicon system (19).

Enhancement of natural killer (NK) cell-mediated cytotoxicity was seen *in vitro* in murine spleen cells, with a 32-62% increase at concentrations of 0.005-0.5 mM. *In vivo* studies confirmed this finding. Mice treated with isatoribine (42-168 mg/kg) showed a significant increase in NK cell cytotoxicity, as indicated by effector:target cell ratios. The highest NK cell activity was seen in mice receiving 168 mg/kg during the initial 24 h after dosing. Tissue localization studies of isatoribine-stimulated NK cells demonstrated induction in the spleen, bone marrow, lungs and peritoneal exudate cells, with maximal activity recorded at 24, 12, 48 and 72 h, respectively, following a single injection. Combined application of IL-2 and isatoribine to murine spleen cells demonstrated a synergistic effect on cytotoxicity, while no significant enhancement of activity was seen when isatoribine was co-applied with interferon alfa or beta. Moreover, isatoribine treatment was associated with rapid and strong production of interferon, and antibodies against interferon alfa and beta antagonized isatoribine-induced NK cell activation and cytotoxicity. Overall, it appears that interferon alfa and beta are involved in the NK cell activation by isatoribine (20, 21). These findings also suggested its potential for the treatment of tumors and viral infections.

Preliminary evaluation in mice showed that isatoribine produced significant protection against Semliki Forest virus infection. When administered at 100 and 200 mg/kg/day i.p. in 2 divided doses (24 and 18 h before virus inoculation), a significantly higher proportion of mice survived (92% vs. 0% in the placebo group). This effect appeared to be due to its immunopotentiating activity as no in vitro antiviral activity was detected (12). Further studies were performed to determine the role of interferon and NK cells in its antiviral activity. Administration of 100-200 mg/kg i.p. isatoribine to mice produced a rapid increase in serum interferon levels, as early as 1 h post-treatment, although the decline was also rapid (6 h post-treatment). A dose-dependent activation of NK cells was also evident in spleen cell assays, with a 2.5-3-fold and 4.4-5.7-fold activation compared to controls at doses of 50 and 100 mg/kg, respectively. In mice with acute Semliki Forest virus infection, an anti-interferon alfa/beta antibody was able to neutralize the antiviral effect of isatoribine. On the other hand, further experiments in mice indicated that the activation of NK cells by isatoribine was not a significant factor in its protection against this virus (22).

The spectrum of isatoribine's antiviral activity and treatment regimens were investigated in rodent models. Administration of isatoribine (25-100 mg/kg/day i.p.) prior to Semliki Forest virus inoculation provided 67-100% protection against lethal infection, while postviral inoculation was ineffective and oral dosing was not effective at doses up to 400 mg/kg. Administration of isatorabine (three doses beginning 48 h before virus inoculation) in mice inoculated i.p. with herpes simplex virus type 2 (HSV-2) significantly increased the mean survival time (11.2-11.8 days *vs.* 9.8 days in controls) and increased the survival rate (50% *vs.* 8% in controls) at doses of 50 and 100 mg/kg i.p. However, the compound failed to improve HSV-2 skin lesions and survival in hairless mice when administered 24 and 18 h before viral inoculation. Single i.p. injections of isatoribine (50 mg/kg) 24 h before viral inoculation provided complete or almost complete protection against San Angelo virus, and complete protection was also obtained at a higher dose (200 mg/kg) given only 2 h prior to virus inoculation. Prophylactic treatment at doses of 50, 100 and 200 mg/kg given as divided doses at 24 and 18 h before virus inoculation also provided significant protection against banzi virus. Moderate but significant protection was seen in mice receiving i.p. doses of 100 and 200 mg/kg/day administered 24 and 18 h prior to vesicular stomatitis virus (VSV) inoculation. Divided-dose regimens of i.p. isatoribine also provided significant protection against encephalomyocarditis virus infections in mice and intranasal coronavirus infections in suckling rats at 50-200 mg/kg/day, suggesting potential against human respiratory viruses, while treatment provided no protection against influenza B virus infection. In mice doses of 50 and 200 mg/kg i.p. provided significant protection against HSV-1, and a dose of 200 mg/kg/day, given in divided doses at 24 and 18 h preinoculation, was protective against murine cytomegalovirus infection (23, 24).

Another study investigated the efficacy of intranasal delivery of isatoribine against intranasal coronavirus and encephalomyocarditis virus infections in mice. Prophylactic treatment with 0.3% and 1% isatoribine solutions was significantly effective at increasing survival in coronavirus-infected rats; treatment 24 and 18 h before virus inoculation produced a substantial increase in cure rate compared to placebo (93% vs. 39% survival), while treatment postinoculation (+4 and +8 h) failed to improve survival rates, although it did improve survival time. Intranasal application of isatoribine provided moderate but insignificant protection against encephalomyocarditis viral infection, although i.p. administration of 100 mg/kg/day at 24 and 18 h before inoculation proved to be more successful. However, intranasal administration did produce a > 300-fold reduction in viral titers in the upper respiratory tract and suppression of spleen and brain viral levels up to day 6 (25).

Isatoribine was evaluated in a murine model of *Phlebovirus* (Bunyaviridae) infection using the hepatotrophic Adames strain of Punta Toro virus. Viral protection was evident at 50-100 mg/kg/day administered 24 and 17 h before inoculation, 25-100 mg/kg/day at –4 and +3 h relative to inoculation, 12.5-100 mg/kg/day at +24 and +31 h relative to inoculation, and 100 mg/kg/day at +36 and +43 h relative to inoculation. Reductions in mortality were associated with reduced liver icterus scores, serum levels of glutamic oxalate transaminase (GOT) and glutamic pyruvate transaminase (GPT), and significantly lower liver and serum viral titers. Further experiments demonstrated that isatoribine enhanced the therapeutic

index of ribavirin by reducing toxicity and enhancing antiviral activity (26, 27).

Toxicity

Toxicity studies in mice revealed an acute LD_{50} of 320 mg/kg when administered as a single i.p. injection, with the 10% lethal dose estimated to be 200 mg/kg. Doses of 200 mg/kg or less were generally well tolerated, especially when administered as divided daily doses. Treatment over 21 days was possible without cumulative toxicity (23).

Clinical Studies

An open-label, dose-escalation proof-of-concept phase I study profiled the effects of i.v. isatoribine in 32 patients with chronic HCV infection. Isatoribine was administered as a 60-80-min i.v. infusion and patients were randomized to receive once-daily doses of 200 (n=4), 400 (n=4), 600 (n=5) or 800 mg (n=12), while others received 400 mg twice daily for 7 days (n=3) or 800 mg twice daily 3 times weekly for 2 weeks (n=4). Once-daily treatment schedules of isatoribine produced a dose-dependent decrease in plasma HCV RNA levels. Significant attenuation was noted with daily doses of 800 mg, displaying similar effects to standard regimens of pegylated interferon alfa-2b plus ribavirin. These effects were observed against all tested viral genotypes, with the largest reductions observed for genotypes 1 and 3. Assays of whole-blood RNA revealed that the expression of two interferon-responsive genes (ISG15 and OAS) was elevated at the end of each treatment schedule, returning to pretreatment levels approximately 1 week following cessation of isatoribine treatment. Circulating markers of immune induction were modestly increased with 800 mg daily, but while IP-10 and neopterin (both linked to endogenous interferon gamma production) levels differed significantly, interferon alfa levels did not increase significantly. At the highest dose administered (800 mg), systemic clearance was approximately 30 l/h, with a C_{max} of approximately 10 μg/ml and an elimination half-life of approximately 2 h; little or no drug accumulation was seen between dosing intervals (24 h). All adverse events were mild or moderate, the most common being insomnia, joint pain, headache and asthenia. The incidence of flu-like symptoms, which are common with direct cytokine therapy, was very low among isatoribine-treated patients (28, 29).

An open-label phase I trial in healthy volunteers was conducted with the oral prodrug ANA-975. This U.K.-based study was designed to evaluate the safety, tolerability and pharmacokinetics of ascending single oral doses of ANA-975 of 400, 800 and 1200 mg. Interim data from this trial indicate that the bioavailability of ANA-975 is virtually complete and conversion to isatoribine in plasma is rapid and effective, providing levels of isatoribine that have been shown to be clinically relevant. ANA-975 is expected to provide the same combination of antiviral effect and tolerability but with the benefit of oral administration. Together with partner Novartis, Anadys expects to initiate a 28-day study of ANA-975 in HCV patients by the end of this year and Anadys also recently received IND clearance from the FDA. ANA-275 is also being explored for its potential in hepatitis B virus (HBV) infection (30-32).

ANA-971 is another prodrug designed to improve the oral bioavailability of isatoribine. In preclinical animal studies, oral administration of ANA-971 resulted in higher levels of isatoribine in the blood compared to oral administration of isatoribine itself, and produced interferon alfa at levels similar to those observed following intravenous dosing of isatoribine. A phase I clinical trial of ANA-971 was initiated in 2004 to assess safety and pharmacokinetics in approximately 30 healthy volunteers following oral administration (33).

Source

Anadys Pharmaceuticals, Inc. (US). Anadys obtained exclusive worldwide rights to isatoribine from Valeant Pharmaceuticals (the former ICN Pharmaceuticals) in 2000. Prodrug ANA-975 is being developed in collaboration with Novartis.

References

1. Goodman, M., Gamson, E.P. (Scripps Clinic). *Immunostimulating 7-deaza-7-oxa- and 7-deaza-7-thia-analogs of 8-substituted-guanine-9-(1'-beta-D-aldoglycosidyl) derivatives compositions and methods*. EP 0343945.

2. Newel 9. Bhopale, G.M., Nanda, R.H. *Emerging drugs for chronic hepatitis C*. Hepatol Res 2005, 32(3): 146-53.

3. Prous Science R&D Backgrounders: *Hepatitis C (online publication)*. Updated October 17, 2005.

4 Heller, T., Rehermann, B. *Acute hepatitis C: A multifaceted disease*. Semin Liver Dis 2005, 25(1): 7-17.

5. Alter, M.J., Kruszon-Moran, D., Nainan, O.V., McQuillan, G.M., Gao, F., Moyer, L.A., Kaslow, R.A., Margolis, H.S. *The prevalence of hepatitis C virus infection in the Unites States, 1988 through 1994*. New Engl J Med 1999, 341(8): 556-62.

6. Flamm, S.L. *Chronic hepatitis C virus infection*. JAMA – J Am Med Assoc 2003, 289(18): 2413-7.

7. Liang, T.J., Rehermann, B., Seeff, L.B., Hoofnagle, J.H. *Pathogenesis, natural history, treatment, and prevention of hepatitis C*. Ann Intern Med 2000, 132(4): 296-305.

8. Pawlotsky, J.-M., McHutchinson, J.G. *Hepatitis C. Development of new drugs and clinical trials: Promises and pitfalls*. Hepatology 2004, 39(2): 554-67.

9. Bhopale, G.M., Nanda, R.H. *Emerging drugs for chronic hepatitis C*. Hepatol Res 2005, 32(3): 146-53.

10. Pawlotsky, J.-M. *Current and future concepts in hepatitis C therapy*. Semin Liver Dis 2005, 25(1): 72-83.

11. Tedaldi, E.M. *New drug targets for HIV and hepatitis C virus coinfection*. CID 2005, 41(1, Suppl. 1): S101-4.

12. Nagahara, K., Anderson, J.D., Kini, G.D., Dalley, N.K., Larson, S.B., Smee, D.F., Jin, A., Sharma, B.S., Jolley, W.B., Robins, R.K., Cottam, H.B. *Thiazolo[4,5-d]pyrimidine nucleosides. The synthesis of certain 3-beta-D-ribofuranosylthiazolo[4,5-d]pyrimidines as potential immunotherapeutic agents*. J Med Chem 1990, 33(1): 407-15.

13. Netea, M.G., van der Graaf, C., Van der Meer, J.W., Kullberg, B.J. *Toll-like receptors and the host defense against microbial pathogens: Bringing specficity to the innate-immune system*. J Leukoc Biol 2004, 75: 749-55.

14. Lee, J., Chuang, T.H., Redecke, V., She, L., Pitha, P.M., Carson, D.A. et al. *Molecular basis for the immunostimulatory activity of guanine nucleoside analogs: Activation of toll-like receptor 7*. Proc Natl Acad Sci USA 2003, 100: 6646-51.

15. Anadys Pharmaceuticals Website – Product candidates.

16. Colic, M., Gasic, S., Vasilijic, S., Pejanovic, V., Jandric, D., Medic-Mijacevic, L., Rakic, L. *A nucleoside analogue, 7-thia-8-oxoguanosine stimulates proliferation of thymocytes in vitro*. Immunol Lett 1999, 69(3): 293-300.

17. Colic, M., Jandric, D., Gasic, S., Vucevic, D., Popovic, P., Medic-Mijacevic, L., Rakic, L. *Immunosine (7-thia-8-guanosine) acts as a cofactor for proliferation of T cells*. Fundam Clin Pharmacol 2000, 14(3): 209-17.

18. Colic, M., Gasic, S., Vucevic, D., Pavicic, L., Popovic, P., Jandric, D., Medic-Mijacevic, L., Rakic, L. *Modulatory effect of 7-thia-8-oxoguanosine on proliferation of rat thymocytes in vitro stimulated with concanavalin A*. Int J Immunopharm 2000, 22(3): 203-12.

19. Fletcher, S., Banh, V., Aust, B., Kerr, B., Averett, D., Steffy, K. *Examination of interferon-induced gene expression by isatoribine, a toll-like receptor 7 agonist and inducer of the innate immune response*. Hepatology 2004, 40(4, Suppl. 1): Abst 1221.

20. Sharma, B.S., Mhaskar, S., Balazs, L., Siaw, M. *Immunomodulatory activity of a novel nucleoside, 7-thia-8-oxoguanosine: I. Activation of natural killer cells in mice*. Immunopharm Immunotoxic 1992, 14(1&2): 1-19.

21. Sharma, B.S., Mhaskar, S., Balazs, L., Jin, A., Smee, D.F. *Immunomodulatory activity of a novel nucleoside, 7-thia-8-oxoguanosine. II. Characterization of induced effector cells and the mechanism of induction*. Immunopharm Immunotoxic 1992, 14(1&2): 21-38.

22. Smee, D.F., Alaghamandan, H.A., Jin, A., Sharma, B.S., Jolley, W.B. *Roles of interferon and natural killer cells in the antiviral activity of 7-thia-8-oxoguanosine against Semliki Forest virus infections in mice*. Antivir Res 1990, 13(2): 91-102.

23. Smee, D.F., Alaghamandan, H.A., Cottam, H.B., Sharma, B.S., Jolley, W.B., Robins, R.K. *Broad-spectrum in vivo antiviral activity of 7-thia-8-oxoguanosine, a novel immunopotentiating agent*. Antimicrob Agents Chemother 1989, 33(9): 1487-92.

24. Smee, D.F., Alaghamandan, H.A., Cottam, H.B., Jolley, W.B., Robins, R.K. *Antiviral activity of the novel immune modulator 7-thia-8-oxoguanosine*. J Biol Resp Modif 1990, 9(1): 24-32.

25. Smee, D.F., Alaghamandan, H.A., Bartlett, M.L., Robins, R.K. *Intranasal treatment of picornavirus and coronavirus respiratory infections in rodents using 7-thia-8-oxoguanosine*. Antivir Chem Chemother 1990, 1(1): 47-52.

26. Smee, D.F., Huffman, J.H., Coombs, J., Huggins, J.W., Sidwell, R.W. *Effects of 7-thia-8-oxoguanosine alone and in combination with ribavirin on Punta Toro virus infections in mice*. Antivir Res 1990, Suppl. I: Abst 153.

27. Smee, D.F., Huffman, J.H., Gessaman, A.C., Huggins, J.W., Sidwell, R.W. *Prophylactic and therapeutic activities of 7-thia-8-oxoguanosine against Punta Toro virus infections in mice*. Antivir Res 1991, 15(1): 229-39.

28. Horsmans, Y., Berg, T., Desager, J.-P., Mueller, T., Schott, E., Fletcher, S.P., Steffy, K.R., Bauman, L.A., Kerr, B.M., Averett, D.R. *Isatoribine, an agonist of TLR7, reduces plasma virus concentration in chronic hepatitis C infection*. Hepatology 2005, 42(3): 724-31.

29. Horsmans, Y., Berg, T., Desager, J.-P., Muller, T., Schott, E., Bauman, L., Steffy, K., Fletecher, S., Banh, V., Liaw, W., Kerr, B., Averett, D. *Isatoribine, a toll-like receptor 7 agonist, significantly reduced plasma viral load in a clinical proof-of-concept study in patients with chronic hepatitis C virus infection*. Hepatology 2004, 40(4, Suppl. 1): Abst 270.

30. *Anadys initiates phase I clinical trial of ANA975, an oral prodrug of isatoribine*. Anadys Pharmaceuticals Press Release 2005, Feb 2.

31. *Anadys Pharmaceuticals announces exclusive collaboration with Novartis to develop and commercialize ANA975 and additional TLR7-based therapeutics for hepatitis C and hepatitis B*. Anadys Pharmaceuticals Press Release 2005, June 2.

32. *Anadys Pharmaceuticals announces FDA acceptance of IND application for ANA975*. Anadys Pharmaceuticals Press Release 2005, Aug 18.

33. *Anadys announces initiation of clinical trial of ANA971, an orally administered prodrug of isatoribine*. Anadys Pharmaceuticals Press Release 2004, Feb 19.