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HCV NS5B polymerase inhibitors 3: Synthesis and in vitro activity of 3-(1,1-dioxo-*2H*-[1,2,4]benzothiadiazin-3-yl)-4-hydroxy-*2H*-quinolizin-2-one derivatives

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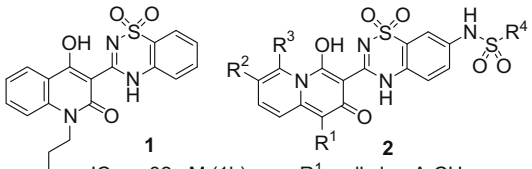
ABSTRACT

3-(1,1-Dioxo-*2H*-[1,2,4]benzothiadiazin-3-yl)-4-hydroxy-*2H*-quinolizin-2-one derivatives as potential anti-HCV drugs targeting NS5B polymerase have been investigated. Their synthesis, HCV NS5B polymerase inhibition, and replicon activity are discussed.

Hepatitis C is a major cause of end-stage liver disease as well as the leading cause of liver transplantations.[1] About 3% of the world's population has been infected with HCV and, of these, 60–80% may progress to chronic liver disease, and 20% of these develop cirrhosis.[2] Thus far, there is no universally effective therapy for all HCV genotypes. The current treatment for patients infected with genotype 1 HCV is 48 weeks of pegylated interferon-α (Peg-IFN-α) and ribavirin (RBV). One of the main antiviral effects from Peg-IFN-α and RBV comes from a boost of the natural immune response. The success rate for achieving a sustained viral response for genotype 1 patients in the US, Europe and Japan is ~40%.[3] The long duration of treatment is difficult for patients to tolerate owing to side effects associated with Peg-IFN-α and RBV that include flu-like symptoms, fatigue, depression, gastrointestinal symptoms, pulmonary effects, and others.[3] These limitations have led to intense interest in the discovery and development of novel compounds that target the viral and host proteins.

HCV NS5B polymerase is an RNA dependent RNA polymerase that resides at the C-terminal domain of a polypeptide of several structural and nonstructural proteins and contains the catalytic machinery responsible for synthesis and replication of the viral RNA.[4] NS5B is essential for the viral replication and has been clinically validated.[6] Along with HCV protease NS3/4A, NS5B is recognized as the most viable protein target for HCV drug discovery.[5–7] Two classes of NS5B inhibitors have been well developed: active site inhibitors such as nucleoside or nucleotide inhibitors that mimic natural polymerase substrates and allosteric inhibitors that bind to less conserved sites outside the active site and impair the enzyme's catalytic efficiency.

Recently, several classes of non-nucleoside allosteric NS5B inhibitors[5–7] have been reported, some of which achieved low nanomolar inhibition in both enzyme and replicon systems. Among the most potent inhibitors are 1,1-dioxo-*2H*-benzothiadiazine compounds, of which compound **1** shown below was one of the first benzothiadiazine compounds found active against HCV NS5B polymerase.[8] Compound **1** binds in a site that is located in the palm domain of the polymerase in the proximity of the active site (see Ref. 8 for a crystal structure of a benzothiadiazine compound bound to NS5B). Recent work from a different institution identified the low nanomolar inhibitors,[9,10] which exhibited a good DMPK profile and showed anti-HCV activity in chimpanzee model.[11]



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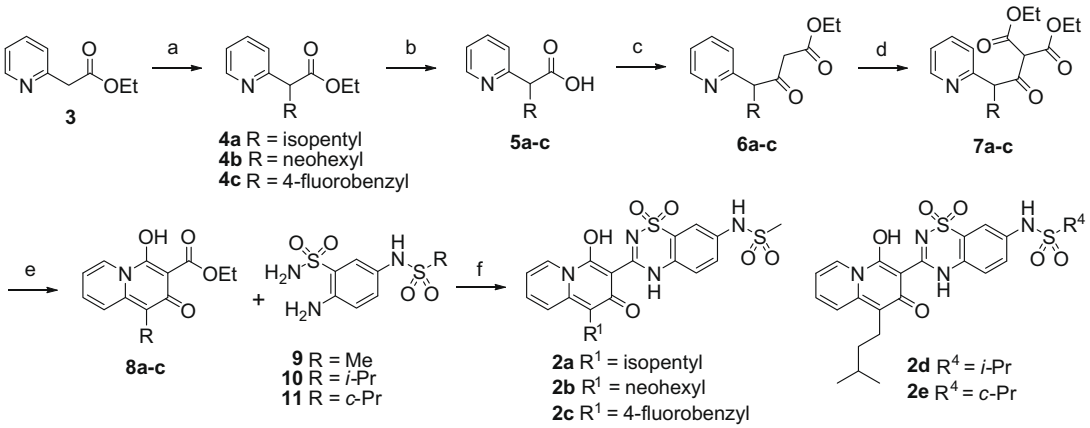


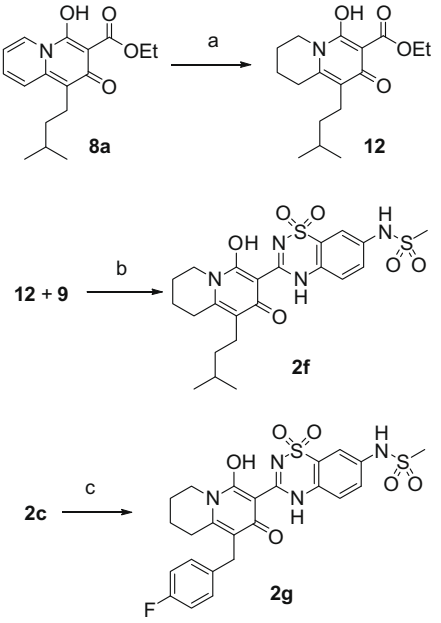
Scheme 1. Reagents and conditions: (a) *i*-Pent-Br, LDA, THF, −78 °C, rt, overnight, 64–70%; (b) NaOH, H_2O, rt, overnight; (c) (i) CDI, THF, rt, 3 h; (ii) $KOCOCH_2COOEt$, $MgCl_2$, TEA, THF, rt, 2 days, 45–55% (2 steps); (d) EtOCOCl, NaH, THF, rt, 2 h; (e) DMSO, 120 °C, 2 h, 25–38% (2 steps); (f) PPSE, 160 °C, 2.5 h, 18–43%.

In search of potent inhibitors of HCV polymerase NS5B at InterMune, we explored several series of benzothiadiazine compounds. In this Letter, we describe synthesis and in vitro anti-HCV activity of compounds **2**.

Target compounds **2a–e** were synthesized, in six steps, as shown in Scheme 1. Alkylation of the ester **3** with isopentyl bromide, neohexyl triflate and 4-fluorobenzyl chloride yielded the alkylated products **4a–c**, respectively, in good yields, which were hydrolyzed to the acids **5a–c**. An attempt to prepare **7a** from **5a** and diethyl malonate via the corresponding acyl chloride or anhydride failed, and a complicated mixture was obtained. A possible reason was that the alpha proton of 5-methyl-2-(pyridin-2-yl)hexanoyl chloride or anhydride may have comparable acidity as the methylene of diethyl malonate, which may have slowed down the desired alkylation and caused side reactions. Finally, **7a–c** were prepared, respectively, by a two-step sequence: conversion of the acids **5a–c** to the ketoacetates **6a–c** by reaction with monoethyl malonate potassium salt and subsequent reaction with ethyl chloroformate. The 1-substituted quinolizinones **8a–c** were prepared, respectively, through cyclization of **7a–c** by heating in DMSO. Condensation of **8a–c** with the methylsulfonamide **9**[9] in polyphosphoric acid trimethylsilyl ester (PPSE)[12] gave the desired benzothiadiazines **2a–c**. Similarly, **2d** and **2e** were prepared by condensation of **8a** with the isopropylsulfonamide **10** and the cyclopropylsulfonamide **11**, respectively.

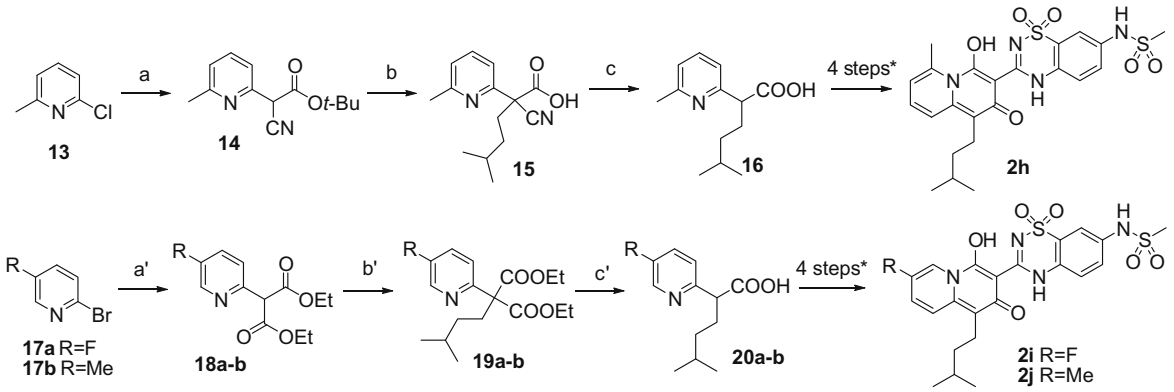
Compound **2f** was prepared from **8a** by a two-step procedure (Scheme 2). Hydrogenation over palladium selectively reduced the left ring of **8a** to give **12** that contains a saturated 6-membered ring. Condensation of **12** with **9** afforded the desired benzothiadiazine **2f**. The hydrogenation was also successfully applied to the quinolizinone–benzothiadiazine compound **2c**, providing **2g** in 40% yield.

Synthesis of **2h–j** is shown in Scheme 3. Reaction of the commercially available **13** with *t*-butyl cyanoacetate in the presence of *t*-BuOK and $Pd(PPh_3)_4$ gave the ester **14**, which was then alkylated with isopentyl bromide. Decarboxylation of **15** followed by the hydrolysis of the cyano moiety under acidic condition yielded the acid **16**, which was subjected to a 4-step conversion described in Scheme 1 (c–f) to afford the desired **2h**. In the similar fashion the reaction of the commercially available **17a** and **17b** with diethyl malonate in the presence of cuprous iodide and cesium carbonate gave **18a** and **18b**, respectively. Alkylation and subsequent decarboxylation of **18a** and **18b** yielded the acids **20a** and **20b**, respectively. Compounds **20a** and **20b** were converted to **2i** and **2j**, respectively, by the 4-step procedure shown in Scheme 1.



Scheme 2. Reagents and conditions: (a) H_2, 10% Pd/C, AcOH, 60 °C, 71%; (b) same as (f) in Scheme 1; (c) H_2, 10% Pd/C, AcOH, rt, 40%.

Benzothiadiazine compounds **2a–j** were tested in HCV NS5B genotype 1b polymerase and replicon assays,[13,14] and the results are presented in Table 1. All the compounds exhibited very potent inhibition of HCV NS5B polymerase, with all IC_{50} values less than 5 nM, which was lower limit imposed by the enzyme concentration. The most active compound in the replicon system was compound **2c** with EC_{50} value of 2.3 nM and CC_{50} value of greater than 100 μM, which translated to a CC_{50}/EC_{50} ratio of more than 40,000. Other four compounds **2a**, **2b**, **2i** and **2j** also exhibited excellent replicon activity with EC_{50} values of 5–17 nM. The rest of the compounds in Table 1 also showed significant replicon activity. R^1 of the structure **2** was selected from a group of alkyls based on the excellent activity of the quinolinone–benzothiadiazine compounds bearing these alkyl groups,[8] however, the activity order in the quinolizinone–benzothiadiazine series in this Letter seems different from that in the quinolinone–benzothiadiazine series. In the quinolizinone–benzothiadiazine series the best R^1 so far was 4-fluorobenzyl group, as shown by the EC_{50} value (2.3 nM) of com-



Scheme 3. Reagents and conditions: (a) N≡CCH_2COOt-Bu, *t*-BuOK, $Pd(PPh_3)_4$, dioxane, 70 °C, overnight, 46%; (b) isopentyl bromide, Cs_2CO_3, DMF, rt, overnight, 70%; (c) HCl, H_2O, 100 °C, overnight, quant.; (a′) $H_2C(COOEt)_2$, picolinic acid, CuI, Cs_2CO_3, dioxane, 100 °C, overnight, 42–48% from **17b**; (b′) isopentyl bromide, K_2CO_3, DMF, 50–60 °C, overnight, 58–74%; (c′) NaOH, EtOH, H_2O, 100 °C, 1 h, 84%. *Four steps include steps c, d, e and f in Scheme 1.

Table 1
Inhibition of HCV NS5B polymerase genotype 1b by compounds **2a**–**2j** and replicon activity

Compound	IC_{50} (μM)	EC_{50} (μM)	CC_{50}[a] (μM)	Compound	IC_{50} (μM)	EC_{50} (μM)	CC_{50}[a] (μM)
2a	<0.005	0.017	30	**2f**	<0.005	0.21	>1
2b	<0.005	0.010	>100	**2g**	<0.005	0.036	>10
2c	<0.005	0.0023	>100	**2h**	<0.005	0.20	>1
2d	<0.005	0.13	>10	**2i**	<0.005	0.005	>1
2e	<0.005	0.057	>10	**2j**	<0.005	0.015	>1

[a] The initial CC_{50} values of **2a**–**j** were either >10 μM or >1 μM, limited by the compound concentrations used, but the CC_{50} values of **2a**–**c** were re-determined at higher compound concentrations.

pound **2c** which is several fold lower than **2a** and **2b** (EC_{50}: 17 and 10 nM) in which R^1 is isopentyl and neohexyl group while the best R^1 was cyclopropylethyl, neohexyl and isopentyl in quinolinone–benzothiadiazine series.[8] Further R^1 optimization may be useful for enhancement of potency as well as CC_{50}/EC_{50} ratio and possibly for improvement of other drug-like properties such as solubility and cell-permeability. The data in Table 1 indicate that methylsulfonamide at the R4 position of the structure **2** is superior to the cyclopropylsulfonamide and isopropylsulfonamide, probably implicating the effect of substituent size. It seems that a small R^2 is well tolerated, as indicated by excellent IC_{50} and EC_{50} values of **2i** and **2j** (R^2 = F or Me). However, compared with **2a**, the methyl group at the R^3 position decreased the replicon activity by more than 10-fold (17 nM for **2a** vs 200 nM for **2h**), indicating the intolerance of a substituent at this position. It is interesting to note that compounds **2f** and **2g** in which the left ring is saturated still exhibited good activity in both NS5B and replicon assays. Overall, the data in Table 1 clearly demonstrate that the quinolizin-2-one-benzothiadiazine ring system is a very promising scaffold for further derivatization and optimization.

In summary, we have successfully built a novel quinolizinone–benzothiadiazine scaffold. Ten compounds based on this scaffold have been synthesized and tested for their anti-HCV activity. All the compounds inhibited HCV NS5B polymerase at less than 5 nM concentration and exhibited good to excellent replicon activity. Particularly, compound **2c** has EC_{50} value of 2.3 nM and its CC_{50}/EC_{50} ratio exceeds 40,000. Further optimization and biological studies are underway and will be reported in the due time.

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14. *HCV replicon assay (EC_{50}, μM)*: A modified assay based on a published method (Vrolijk, J. M.; Kaul, A.; Hansen, B. E.; Lohmann, V.; Haagmans, B. L.; Schalm, S. W.; Bartenschlager, R. *J. Virol. Methods* **2003**, *110*, 201) was used. Exponentially growing Huh-7 cells stably transfected with luc/neo ET replicon were maintained in DMEM media supplemented with 10% fetal

calf serum and seeded at a density of 5×10^3 cells/well in white 96-well plates. Compounds were dissolved in DMSO, diluted in DMSO in a serial fashion to create an appropriate range of concentrations, and added to cells approximately 24 h after plating. The final DMSO concentration in the cell plate was 1%. After 46–50 h exposure, the media was discarded from the assay plate and the cell monolayers were lysed by addition of 100 μL of either BrightGLO (Promega) or ATPlite reagent (PerkinElmer) with incubation at 20 °C for 2 min on an orbital shaker. Following incubation, luminescence was assessed on a SpectraMax M5 plate reader (Molecular Devices). Plots of luminescesce versus log compound concentration were fit to a 4-parameter logistic equation to determine EC_{50} and CC_{50} values.