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HCV NS5B polymerase inhibitors 2: Synthesis and in vitro activity of (1,1-dioxo-*2H*-[1,2,4]benzothiadiazin-3-yl) azolo[1,5-*a*]pyridine and azolo[1,5-*a*]pyrimidine derivatives

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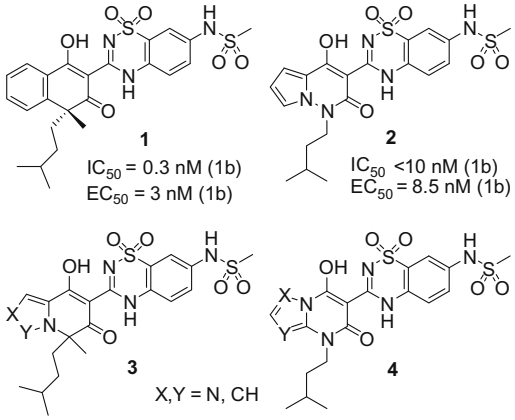
ABSTRACT

(1,1-dioxo-*2H*-[1,2,4]benzothiadiazin-3-yl) azolo[1,5-*a*]pyridine and azolo[1,5-*a*]pyrimidine derivatives have been investigated as potential anti-HCV drugs. 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 (48 weeks for genotype 1) 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 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.



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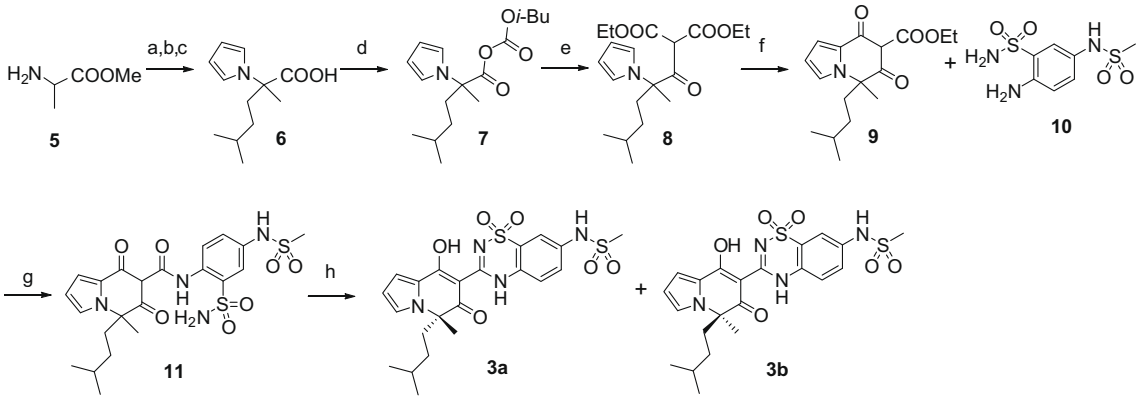


Scheme 1. Reagents and conditions: (a) 2,5-$(MeO)_2$THF, KOAc, AcOH, H_2O, reflux, quant. (crude); (b) *i*-Pent-Br, LDA, THF, −78 °C, rt, overnight, 22%; (c) LiOH, MeOH, H_2O, rt, 6 h, 59%; (d) *i*-BuOC(O)Cl, TEA, DCM, 0 °C, 4 h, 82%; (e) diethyl malonate, NaH, THF, rt, 2 h, 57%; (f) CH_3SO_3H, rt, overnight, 29%; (g) toluene, reflux, 2 h; (h) PPSE, 160 °C, 3 h, 15% (two steps).

Recently, several classes of non-nucleoside allosteric NS5B inhibitors have been reported,[5–7] some of which achieved low nanomolar inhibition in both enzyme and replicon assays. Among the most potent NS5B inhibitors are 1,1-dioxo-*2H*-benzo[1,2,4]thiadiazines, of which compounds **1** and **2** shown above exhibited promising activities against HCV NS5B polymerase.[8,9] Compound **1** also exhibited a good DMPK profile achieving high plasma and liver concentration and showed anti-HCV activity in chimpanzee model.[10]

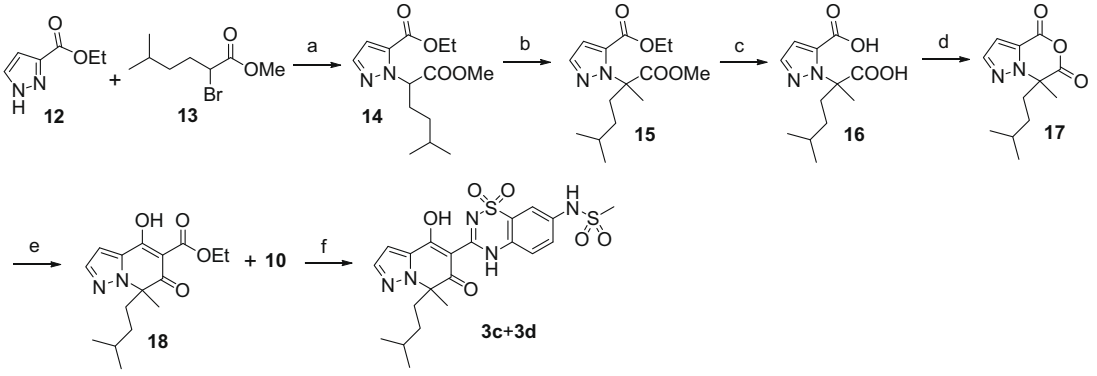
In search of potent allosteric inhibitors of HCV NS5B polymerase at InterMune, we explored several series of benzothiadiazine compounds. In this report we describe synthesis and in vitro anti-HCV activities of azolo[1,5-*a*]pyridine-benzothiadiazines **3** and azolo[1,5-*a*]pyrimidine-benzothiadiazines **4**. An X-ray crystal structure of **4a** bound to HCV NS5B polymerase is also presented.

Synthesis of pyrrolo[1,5-*a*]pyridine-benzothiadiazine **3a** and **3b**[11] is shown in Scheme 1. 2-Aminopropanoic acid (**5**) was reacted with 2,5-dimethoxytetrahydrofuran in the presence of KOAc, followed by alkylation and hydrolysis, to yield the acid **6**. Treatment of **6** with isobutyl chloroformate yielded the anhydride **7**. Reaction of **7** with diethyl malonate yielded **8**, which was cyclized to afford the key intermediate **9**. Condensation of **9** with the 2-aminobenzenesulfonamide **10**[8] gave the coupling product **11**, which was cyclized in polyphosphoric acid trimethylsilyl ester (PPSE) according to a published procedure.[12] The racemic mixture of **3a** and **3b** was separated using a chiral supercritical prep LC[13] and their stereochemical assignment achieved by X-ray crystallography.[14]

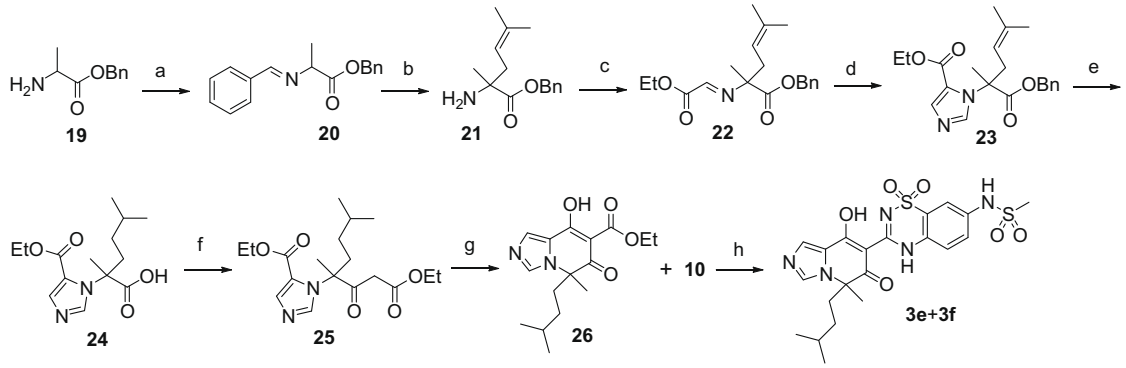
Synthesis of **3c** and **3d** is shown in Scheme 2. Alkylation of **12** with the bromide **13** yielded two N-alkylated products, of which **14** was isolated as the minor product. C-Methylation of **14** and subsequent hydrolysis gave the acid **16**, which was converted to the anhydride **17** by treatment with ethoxytrimethylsilylacetylene. The crude **17** was used directly for reaction with diethyl malonate. The resulting **18** was condensed with **10** to give the racemic mixture of **3c** and **3d**.

Synthesis of **3e** and **3f** is shown in Scheme 3. Benzyl 2-aminopropionate (**19**) was reacted with benzaldehyde to give the Schiff base **20**. Alkylation of **20** and subsequent acidic hydrolysis yielded **21**, which was condensed with ethyl glyoxylate to form **22**. Treatment of **22** with tosylmethylisocyanide (TosMIC) in the presence of base yielded the imidazole derivative **23**. Hydrogenation/hydrogenolysis of **23** over palladium reduced 2-methylbutenyl group to isopentyl and deprotected benzyl group concomitantly to give the acid **24**. The β-ketoacetate **25** was prepared by conversion of **24** to the acyl chloride and subsequent reaction with ethyl acetate in the presence of LiHMDS. Compound **25** was cyclized in the presence of DBU and the resulting **26** was condensed with **10** to afford the racemic mixture of **3e** and **3f**.

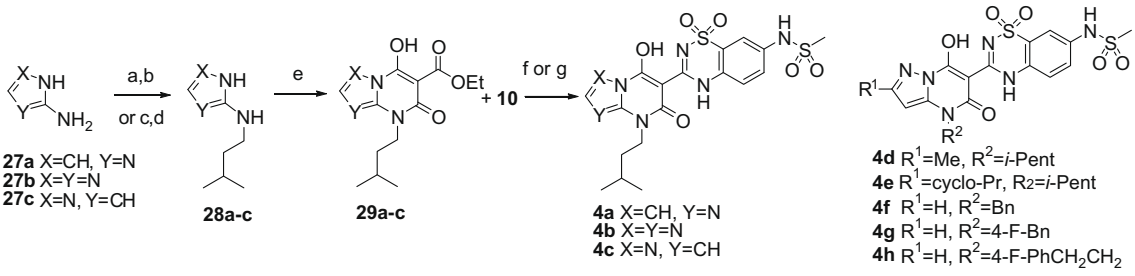
Synthesis of the azolo[1,5-*a*]pyrimidines **4a–h** is shown in Scheme 4. Compounds **27a–b** were converted to the corresponding secondary amines **28a–b**, respectively, in moderate yields by reaction with isopentanoyl chloride and subsequent treatment with alane–dimethylethylamine complex while **27c** was converted to **28c** by reaction with isovaleraldehyde and subsequent reduction. Condensation of **28a–c** with triethyl methanetricarboxylate and the following spontaneous cyclization gave **29a–c**, respectively, in moderate yields. Condensation of **29b–c** with **10** in PPSE afforded **4b–c**, respectively, in low yields while condensation of **29a**



Scheme 2. Reagents and conditions: (a) K_2CO_3, NMP, 80 °C, 2 h, 11%; (b) MeI, LiHMDS, THF, −78 °C, 4 h, then rt, 74%; (c) NaOH, EtOH, H_2O, reflux, 4 h, 90%; (d) TMS–OEt–acetylene, $ClCH_2CH_2Cl$, 70 °C, 3 days; (e) diethyl malonate, NaH, DMA, 120 °C, 4 h, 11% (two steps); (f) PPSE, 160 °C, 1.5 h, 13%.



Scheme 3. Reagents and conditions: (a) PhCHO, 4 Å MS, $CHCl_3$, rt, 24 h, quant; (b) i. 3,3-dimethylallyl bromide, LiHMDS, THF, −78 °C to rt, 16 h, ii. 1 M HCl/H_2O, rt, 2 h, 80%; (c) EtOC(O)C(O)H, 4 Å MS, THF; (d) TosMIC, LiHMDS, THF, −78 °C to rt, 45% (two steps); (e) H_2, Pd/C, THF, rt, quant; (f) i. $(COCl)_2$, cat. DMF, THF, rt, 5 h; ii. EtOAc, LiHMDS, THF, −78 to −20 °C, 1 h, 44%; (g) DBU, toluene, 50 °C, 4 h, quant; (h) PPSE, 140 °C, 2 h, 45%.



Scheme 4. Reagents and conditions: (a) *i*-BuO(CO)Cl, pyridine, rt, 16 h; (b) alane–NMe_2Et complex, THF, 50 °C, 2 d for **27a–b**; (c) isovaleraldehyde, THF, AcOH, 4 Å MS, rt, 16 h; (d) $NaBH_4$, rt, 3 h, for **27c**; (e) $CH(COOEt)_3$, toluene, Microwave, 120–130 °C, 30 min; (f) PPSE, 140 °C, 2 h, for **29b–c**; (g) i—DMF, 110 °C, 10 h; ii—DBU, pyridine, 120 °C, 16 h, for **29a**.

with **10** in DMF with DBU gave **4a** in low yield. Compounds **4d–h** were prepared using the same procedures as described for **4c**.

We obtained a co-crystal structure of compound **4a** in complex with HCV NS5B polymerase genotype 1b Δ21N-His-tagged at 1.9 Å resolution (Fig. 1).[15] The ligand binds to the enzyme at an allosteric site located in the palm domain of the polymerase, similarly to other benzothiadiazines reported in the literature[9,16] The dihedral angle between the azolopyrimidine ring and the benzothiadiazine moiety is close to 22°. The ligand core is anchored in the binding site via several hydrogen bonds and water-mediated contacts with the protein. An ordered water molecule participates in a network of polar interactions with one of the sulfonamide oxygens of the benzothiadiazine and the backbone amide nitrogen of Ser556, as well as the side chain OH of Ser288. An additional ordered water molecule bridges interactions between the enolic OH of the pyrimidone ring with the backbone amide nitrogen of Gly449. This water molecule is located within 3.3 Å of the second benzothiadiazine sulfonamide oxygen. The methyl sulfonamide substituent on the benzothiadiazine ring is involved in additional polar interactions with the protein. The amide NH of this group is within hydrogen bonding distance of the Asp318 side chain, while one of the sulfonamide oxygens is within 3 Å or less of two hydrogen-bond donors on the protein: the backbone NH of Asp318 and the side-chain N-atom of Asn291. The isopentyl substituent on the pyrimidone ring

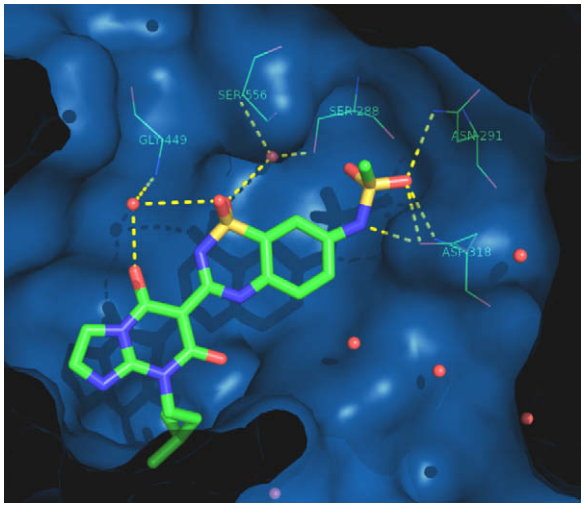


Figure 1. Co-crystal X-ray structure of compound **4a** bound to HCV NS5B polymerase at 1.9 Å resolution. Hydrogen bonds and polar interactions discussed in the text are shown as dashed lines. Figure rendered with PyMOL.

Table 1
Inhibition of HCV NS5B polymerase genotype **1b** and replicon by compounds **3a–f** and **4a–h**

Compound	IC_{50} (μM)	EC_{50} (μM)	CC_{50} (μM)
3a + 3b	<0.005	0.23	>100
3a	<0.005	0.15	19.5
3b	0.15	4.9	170
3c + 3d	<0.005	1.1	>100
3e + 3f	<0.005	6.6	>100
4a	<0.005	0.55	>1
4b	<0.005	6.7	>100
4c	<0.005	0.55	>100
4d	<0.005	1.7	>100
4e	0.009	>20	>100
4f	<0.005	4.0	>100
4g	<0.005	12	>100
4h	0.018	>100	>100

occupies a deep hydrophobic pocket formed by the side chains of Pro197, Leu384, Met414, Tyr415, and Arg200.

Benzothiadiazine compounds **3a–f** and **4a–h** were tested in HCV NS5B genotype 1b polymerase and replicon assays.[17,18] As can be seen from Table 1, optically pure **3a** and the racemic mixtures **3a** + **3b**, **3c** + **3d**, and **3e** + **3f** all showed very potent NS5B inhibition with IC_{50} < 5 nM, which was the lower limit imposed by the enzyme concentration. However, the compounds exhibited significant difference in replicon activity. EC_{50} value of the racemic **3a** + **3b** was 0.23 μM, which is 5-fold and 29-fold more active than the racemic mixtures **3c** + **3d** (EC_{50}: 1.1 μM) and **3e** + **3f** (EC_{50}: 6.6 μM), respectively. Why additional nitrogen in the azole ring has detrimental effect on replicon activity remains unclear and needs further investigation. Compounds **4a–d** and **4f–g** all exhibited potent NS5B inhibition with IC_{50} values less than 5 nM. Compounds **4a** and **4c** exhibited submicromolar replicon activity while other compounds in **4** series mostly had EC_{50} values in low micromolar range. Compounds **4e** and **4h** exhibited lower potency in both enzyme and replicon assays. Lower potency of **4h** compared to **4g** is probably due to the introduction of the bulky 4-fluorophenethyl group in place of the smaller 4-fluorobenzyl moiety while lower potency of **4e** compared to **4d** is likely due to replacement of the methyl group on the azole ring by a larger cyclopropyl group.

In summary, four novel azolo[1,5-*a*]pyridine and azolo[1,5-*a*]pyrimidine scaffolds have been described. Most of the compounds derived from these scaffolds demonstrated very potent NS5B inhibition (<5 nM) and exhibited promising replicon potency reaching submicromolar EC_{50} values as exemplified by compounds **3a**, **4a** and **4c**. These scaffolds have been selected for further derivatization and optimization, which are underway.

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18. *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 (Perkin–Elmer) 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 luminescence versus log compound concentration were fit to a 4-parameter logistic equation to determine EC_{50} and CC_{50} values.