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Parallel synthesis of pteridine derivatives as potent inhibitors for hepatitis C virus NS5B RNA-dependent RNA polymerase

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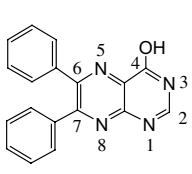
Abstract—From compound library screening using an HCV NS5B RNA-dependent RNA polymerase enzymatic assay, we identified a pteridine hit compound with an IC_{50} of 15 μM. Our SAR studies were focused on the different groups at the 6- and 7-positions, substitutions at the 4-position, and replacement of N_1 or N_3 with carbon in the pteridine ring. We found that NH or OH at 4-position is critical for the inhibitory activity. Furthermore, a hydrophobic substituent at the 4-position may help compounds permeate through the cell membrane.

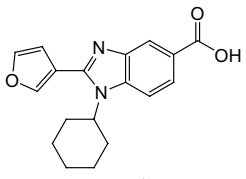
It is estimated that 170 million people worldwide are infected with hepatitis C virus (HCV) including 4 million in the United States.[1] Within 20 years of infection, a significant proportion of them will develop cirrhosis and end-stage liver diseases such as hepatocellular carcinoma.[2] Current therapies based on combinations of pegylated interferons and ribavirin have limited efficacy in a significant proportion of patients and are associated with some severe side effects.[3] Thus there is an urgent need to develop more efficacious and safer therapeutics for the treatment of chronic HCV infection and associated liver diseases.[4]

The HCV NS5B RNA-dependent RNA polymerase (RdRp) is a central enzyme in the replication of the viral genome and has since become a target of choice for screening and design of small molecule inhibitors for viral replication interference.[5,6]



1a

IC_{50} = 15 μM



1b

IC_{50} = 1.6 μM

From a high throughput screening of compound libraries by using an HCV NS5B RdRp enzymatic assay,[7] we identified an interesting hit **1a** with an IC_{50} of 15 μM. This compound possesses a pteridine pharmacophore. Although it is not very potent, it bears certain resemblance to a benzimidazole 5-carboxylate NS5B RdRp inhibitor **1b** discovered by Boehringer Ingelheim.[8] Compared to the hit **1b**, compound **1a** is less negatively charged and may have advantage to possess better cellular permeability. Since the hit **1b** has been successfully optimized to achieve nanomolar potency,[8] we attempted to optimize compound **1a** through systematic SAR studies. In this communication, we report parallel synthesis of pteridine derivatives of **1a** and evaluation of their inhibitory activities against HCV NS5B RdRp.

Most of the compounds described in this study were prepared according to Scheme 1. Through the condensation of 4,5-diaminopyrimidone **2** with commercially available diketones (**3a**–**l**) in refluxing acetic acid in the presence of sodium acetate, compounds **4a**–**l** were obtained as solids in 50–60% yields. After chlorination with $POCl_3$, compounds **4a**–**l** were converted into their corresponding chlorinated derivatives **5a**–**l** with 50–70% yields, which were then used as key intermediates for the library synthesis. Through a parallel synthetic strategy, compounds **5a**–**l** were reacted, respectively, with

Keywords: HCV; Inhibitor; NS5B; Pteridine.
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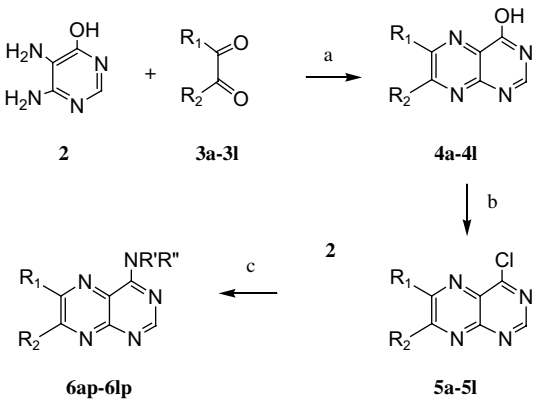


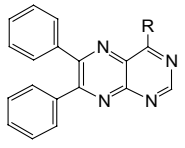
Scheme 1. Reagents and conditions: (a) **3a–l**, compound **2**, AcOH, AcONa, 150 °C, 2 h; (b) **4a–l**, $POCl_3$, 75 °C, 1 h; (c) **5a–l**, amines, EtOH, 70 °C, 2 h.

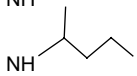
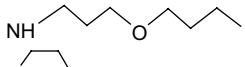
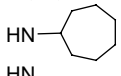
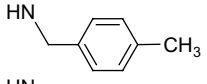
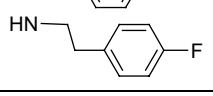
different amines to provide compounds **6ap–lp**. Compounds **1aa–ah** were obtained from the same synthetic strategy. Their structures were confirmed by ESMS and ^1H NMR spectra. All the compounds for biological assays are more than 90% pure by (HPLC).

The compounds described in this study were evaluated for inhibitory activity against HCV NS5B RdRp using a published procedure.[7] Compounds were first dissolved into DMSO, and then transferred into the assay buffer. Each compound was tested in duplicate. IC_{50} (inhibitor concentration for 50% inhibition) was determined for each compound with a standard deviation typically <10%.

As shown in Table 1, substitution at the 4-position of pteridine of **1a** with a bulky amino group (**1ac**, **1ad**, **1ag** and **1ah**) was not tolerated for activity. However, the mid-size alkyl amino derivative **1ab** was 2-fold more potent than compound **1a**. Further reduction in the size of the amino group (**1aa**) results in a slight decrease of activity. From this initial SAR, it seems that a free NH is required for maintaining activity, as substitution by a tertiary amine, as in the case of compound **1ae**, results in a total loss of activity.

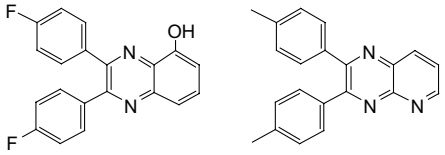
Table 1. SAR at the 4-position of pteridine of compound **1a**

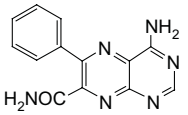


Compounds	R	IC_{50} (μM)
1aa		16
1ab		6
1ac		>100
1ad		87
1ae		>100
1af		29
1ag		>100
1ah		>100

After the series of modifications on the 4-position, we then evaluated the modifications at the 6,7-diaryl moiety. The effects of the modifications are summarized in Table 2. Substitutions at the *para*- or *meta*-position on either ring with an electron donating group (**4b** and **4d**) resulted in a considerable loss of activity. Introduction of a fluorine atom at the *para*-position of the aryl group (**4e**) increased the potency about 30-fold with respect to compound **1a** with an IC_{50} of 0.5 μM. Surprisingly, introduction of a bromine atom at the *para*-position of the aryl part (**4c**) dropped the potency by 2-fold, suggesting that NS5B RdRp can not tolerate a big substituent at the position. Introduction of a chlorine atom at the *ortho*-position (**4h**) also resulted in loss of the activity, indicating that a small group at the *para*-position is optimal for activity. When polar heterocycle groups such as 2-pyridine or 2-furan replaced the phenyl groups (**4i** and **4j**), the compounds lost inhibitory activity, suggesting that neutral substituents are preferred at 6,7-positions. Loss of activity for compounds **4k** and **4l** indicated that the existence of both aryl groups is required, and the enzyme NS5B RdRp does not prefer the planar conformation of **4l**.

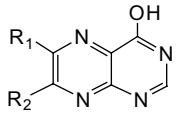
We also examined the activity for compounds **4m**, **4n** and **4o**, in which the N_3 and/or N_1 in the pteridine ring was replaced by carbon atom or one aryl group was replaced by $CONH_2$ group to assess the importance of the N_1 and N_3 moieties and the aryl part. Interestingly, these compounds possess no activity against NS5B RdRp, highlighting the importance of the presence of the N_1 and N_3 in pteridine pharmacophore, and confirmed the importance of the diaryl groups for the potency.

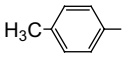
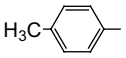
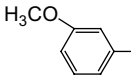
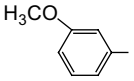
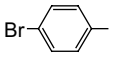
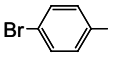
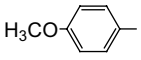
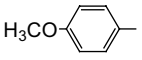
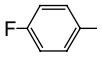
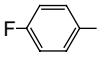
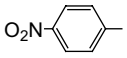
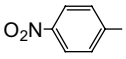
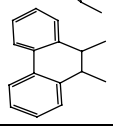




It is of interest to examine whether the features of *p*-fluoro aryl scaffold (**4e**) and 4-alkylamino substitution could be combined to further improve the potency. The inhibitory activities of the 4-position amino derivatives for compound **4e** are listed in Table 3. Again, bulky amino groups (**6ec**, **6ed**, **6eg**, **6eh**, **6ej**, **6eo** and **6ep**) reduced the activity. Also, consistent with the SAR

Table 2. SAR at the 6-, 7-disubstitution of pteridine of compound **1a**



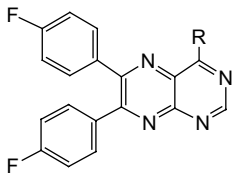
Compounds	R_1	R_2	IC_{50} (μM)
4a			>100
4b			>100
4c			30
4d			>100
4e			0.5
4f			23
4g			30
4h			>100
4i			>100
4j			58
4k			>100
4l			>100

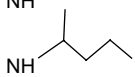
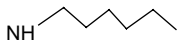
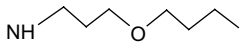
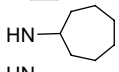
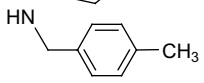
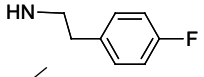
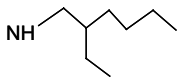
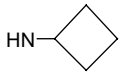
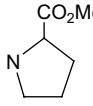
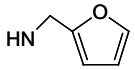
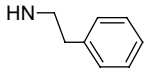
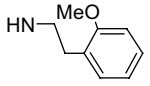
based on compound **1a**, lack of an NH group (**6ee**, **6ei** and **6em**) was detrimental to the activity, emphasizing the importance of a free NH group there. In this series, compounds with a mid sized amino group (**6eb)** and nonplanar cyclohexyl amino group (**6el**) gave the best IC_{50} (1.5 and 1.3 μM). However, they are still less potent than **4e**.

Similar alkyl amine modifications for other disubstituted pteridine analogues were also made, and the resulting compounds did not show much improvement for the inhibitory activity.

We further evaluated the compounds with IC_{50} under 5 μM for cellular activity using a cell-based HCV subgenomic replicon assay. Compound **4e**, which was the most active in the NS5B RdRp assay (IC_{50} = 0.5 μM), showed an EC_{50} of 90 μM in the replicon assay. However, its alkyl aminated analogue **6el** showed a much better activity in the replicon assay with an EC_{50} of 18 μM and no toxicity up to 250 μM. This result indicates that the partially negatively charged 4-hydroxyl in **4e** may be problematic for cellular permeability. However, introduction of a hydrophobic amino group at the 4-position may overcome this problem.

Table 3. SAR at the 4-position for compound **4e** with the IC_{50} of 0.5 μM



Compounds	R	IC_{50} (μM)
6ea		2.9
6eb		1.5
6ec		>100
6ed		73
6ee		68
6ef		3.2
6eg		9.6
6eh		26
6ei		21
6ej		10
6ek		2.1
6el		1.3
6em		51
6en		2.9
6eo		13
6ep		80

In conclusion, from the compound screening using an HCV NS5B RdRp enzymatic assay, we identified a hit compound **1a** with an IC_{50} of 15 μM. We explored the structure–activity relationship for this class of inhibitors. Introducing a fluorine atom at the *para*-position of the aryl part increases the potency dramatically, but addition of a bulky group at the *para*-, *ortho*- or *meta*-position on the aryl group are not tolerated. Small alkyl amines at the 4-position of the pteridine can maintain or increase the potency. NH or OH at the 4-position and presence of N_1 and N_3 in the pteridine ring is critical for activity. Introduction of a hydrophobic amino group at the 4-position may help compounds gain permeability

and display better EC_{50} in the cell-based replicon assay. Current studies are focusing on elucidation of the mechanism of action of these pteridine inhibitors against HCV NS5B RdRp and further improvement of their enzymatic and cellular potency through structure-based drug design efforts.

References and notes

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