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Synthesis and anti-HCV activity evaluation of anilinoquinoline derivatives

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ABSTRACT

Hepatitis C virus (HCV) infection is a main cause of chronic liver disease, leading to liver cirrhosis and hepatocellular carcinoma (HCC). The objective of our research was to develop effective agents against viral replication. Here, we have synthesized a series of anilinoquinoline derivatives. Based on a cell-based HCV replicon system, we observed that 2-(3′-nitroanilino)quinoline (**18**) exhibited anti-HCV activity with a 50% effective concentration (EC_{50}) value of 7 μM and a selective index (SI) value of 10. In addition, compound **18** possessed the inhibitory effect on HCV NS3/4A protease activity. Therefore, we concluded that the compound **18** possessed a potent activity against HCV replication and could provide as a new lead compound as anti-HCV inhibitor.

Hepatitis C virus (HCV) is an enveloped virus that is classified in the hepacivirus genus of the *Flaviviridae* family.[1] It has a 9.6-kb positive-single strand RNA genome that comprises an open reading frame (ORF) flanked by highly structured 5′ and 3′ untranslated regions (UTRs).[2] The virus RNA genome encodes a polyprotein, which is posttranslationally processed by host and virus proteases into mature proteins, including structural proteins (C, E1, E2, and p7) and nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B).[3] Among the nonstructural proteins, NS3/4A protease has been considered as a prime target to eradicate HCV infection due to possessing multiple functions, including RNA helicase activity for catalyzing the unwinding of RNA–RNA and proteolytic activity for post-translational processing of polyprotein in viral replication.[4] HCV is a worldwide infectious pathogen that causes chronic liver diseases, including hepatic fibrosis, hepatic cirrhosis and hepatocellular carcinoma (HCC).[5] At present, medical treatment of HCV-infected patients is a combination of pegylated interferon-α (IFN-α) and the nucleoside analog ribavirin. However, the therapy usually involves adverse side effects and yields a limited sustained virological response (SVR) of approximately 50% for predominant genotype 1 infected patients.[6,7] Furthermore, there is no available vaccine or specific agents against HCV infection. Currently, novel agents targeting HCV replication are under investigation through the replicon system, which is based on Huh7 hepatoma cell lines containing stably replicating HCV subgenomic RNA.[8] A number of potent anti-HCV agents have been developed for targeting HCV RNA replication, protease, and RNA polymerase activity.[4] Nevertheless, in addition to the dependence of therapeutic effectiveness on virus genotype, drug resistance is an inevitable problem due to the high mutation rate of HCV and its rapid replication.[9,10] Therefore, it is extremely desirable to further develop new, safer and even more effective agents against HCV infection and resistance emergence.

Since the natural products and their derivatives play important roles in drug discovery, most of the synthetic and semi-synthetic compounds and theirs pharmacophores were designed from natural products.[11] Studies have shown that quinoline derivatives had diverse of biological activities, including antifungal, antibacterial, and antiplatelet activities.[12–15] Besides, the 2-phenylquinoline derivative (**1**) were consists of a large number of antitumor compounds.[16–18] In recent years, increased attention has been devoted to the antiviral activity of quinoline derivatives. The naturally occurring quinolone alkaloid, buchapine (**2**), and its synthetic derivative (**3**) exhibited potent inhibitory activities against HIV.[19] Similarly, a 4-hydroxyquinolon-3-yl-benzothiadiazine derivative (**4**) was evaluated as an inhibitor of genotype 1 HCV polymerase and also demonstrated good antiviral activity[20] (Fig. 1). Although there are many studies that reported good antiviral activity for the non-nucleotide inhibitors of the heterocyclic backbone, to date,

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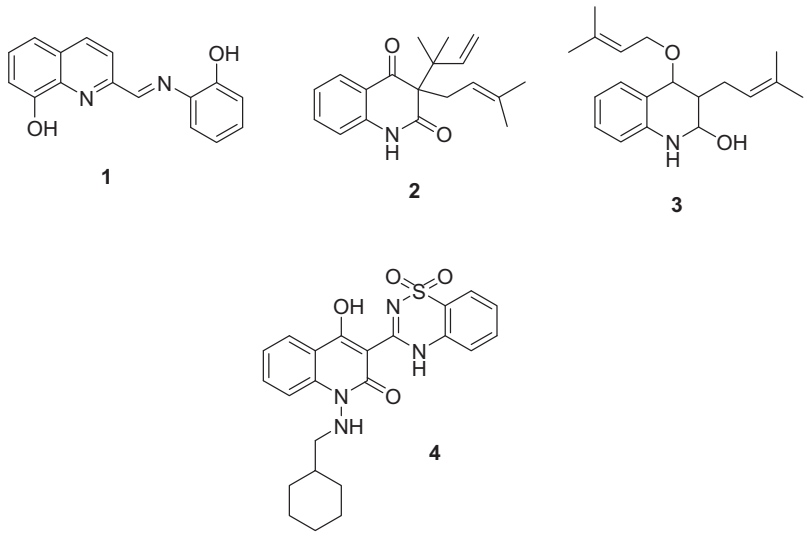
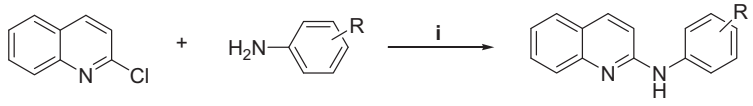


Figure 1. Chemical structure of quinoline derivatives.

there has been no research on the anti-HCV activity of quinoline derivatives.[21–36] Therefore, we were interested in designing and synthesizing an aniline moiety incorporated into a natural quinoline pharmacophore to generate a new anilinoquinoline lead compound. In this study, we examined the anti-HCV activity of synthesized anilinoquinoline derivatives. Quantitative RT-PCR and western blot analyses were performed to quantify the intracellular HCV RNAs and protein expression, respectively.

The synthesized series of anilinoquinoline derivatives was prepared by simple amination. Reaction of the starting material, 2-chloroquinoline (**5**), with various anilines in the presence of ethanol produced **6–23** with overall yields of 34–95%. The preparation of anilinoquinoline derivatives is described in Scheme 1. Designed series of compounds **6–23** were characterized by ^{1}H NMR, ^{13}C NMR, IR spectra, and HRMS techniques, and their purity by element analysis.

To examine the effects of various anilinoquinoline derivatives on HCV replication, Huh7 cells harboring the HCV subgenomic replicon,[8] designated Ava5 cells, were treated with anilinoquinoline derivatives at concentrations of 0.5–150 μM. After three days, total cellular RNAs of compound-treated cells were subjected to quantitative RT-PCR (RT-qPCR) with specific primers corresponding to HCV RNA. In parallel, cytotoxicity was determined by the MTS assay. The results for the anilinoquinoline derivatives are summarized in Table 1. Among them, compounds **6**, **11**, **18**, **19**, **20**, **21**, and **23** exhibited anti-HCV activity at concentrations <30 μM. Compounds **17**, **18**, **20**, and **22** were found to be less cytotoxic (>70 μM). Based on the above CC_{50} (concentration of the compound at which cell viability is reduced by 50% when compared to the mock control, 0.1% DMSO-treated cells) and EC_{50} (concentration of the compound at which HCV RNA level is reduced by 50% when compared to the mock control) results, the selective



6, R = H
7, R = 4'-Me
8, R = 2', 4'-diMe
9, R = 3', 5'-diMe
10, R = 2', 6'-diMe
11, R = 4'-Cl, 6'-Me
12, R = 4'-Cl
13, R = 2'-Cl, 4'-Br
14, R = 2'-Br
15, R = 4'-OMe
16, R = 3', 5'-diOMe
17, R = 3', 4', 5'-triOMe
18, R = 3'-NO_2
19, R = 4'-NO_2
20, R = 3'-NO_2, 4'-OH
21, R = 4'-CN
22, R = 4'-CO_2Et
23, R = 3'-OCH_2Ph

Scheme 1. Reagent and condition: (i) EtOH, reflux (34–95%).

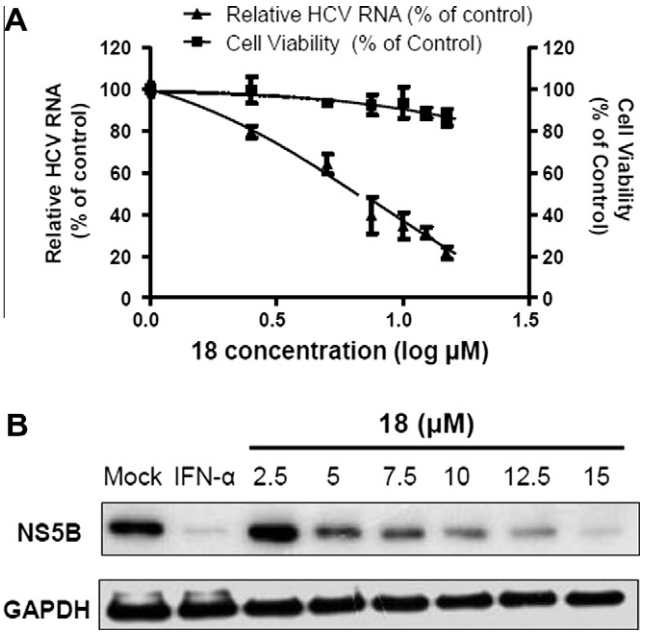


Figure 2. Inhibition of HCV replication and protein expression in Ava5 cells by compound **18**. Ava5 cells were incubated with compound **18** at the indicated concentration (from 2.5 to 15 μM). The non-linear regression graphs and an effective concentration of 50% inhibition (EC_{50}) was calculated by the GraphPad Prism5.0 program (Version 5.0, GraphPad Software, Inc. La Jolla, CA). Treatment with 0.1% DMSO served as a mock control. (A). After three days of incubation, total RNA was extracted from the Ava5 cells to quantify HCV RNA levels by RT-qPCR. HCV RNA expression was normalized by cellular GAPDH mRNA (left axis). Cell viability was performed simultaneously by the MTS assay (right axis). The results are expressed as the means ± standard deviations (error bars) of triplicate experiments. (B). After four days of incubation, cell lysates were extracted and analyzed by western blotting with anti-NS5B and anti-GAPDH antibody (a loading control). Treatment with 100 U/ml of interferon-α (IFN-α) served as positive control of inhibition of HCV protein synthesis.

Table 1
Inhibition of HCV by anilinoquinoline derivatives

Compds	HCV		
	CC_{50}[a] (μM)	EC_{50}[b] (μM)	SI[c]
6	45	23	1.96
7	45	43	1
8	35	35	1
9	31	31	1
10	40	40	1
11	43	12.5	3.4
12	60	53	1.1
13	48	45	1
14	61	60	1
15	46	43	1
16	33	33	1
17	77	25	3
18	71	7	10
19	48	20	2.4
20	84	21.7	3.8
21	50	14.3	3.5
22	147	50	2.9
23	47	15.1	3.1

[a] Concentration of the compound at which cell viability was reduced by 50% when compared to the 0.1% DMSO-treated cells, which served as a mock control.
[b] Inhibitory concentration that reduced viral replication by 50%.
[c] The selective index was calculated as the ratio of CC_{50} versus EC_{50}.

index (SI; CC_{50}/EC_{50}) values for **11**, **17**, **18**, **20**, **21**, and **23** were greater than those for the other compounds. The results indicated that 2-(3′-nitroanilino)quinoline (**18**) displayed the best SI value compared to the other compounds in terms of anti-HCV activity, with a SI value of 10. As shown in Figure 2A, a dose-dependent reduction of HCV RNA levels by compound **18** was also observed, which exhibited an EC_{50} value of 7 ± 0.3 μM, as normalized by cellular GAPDH mRNA. To further clarify the inhibitory effect of compound **18** on the synthesis of viral protein, cell lysates were

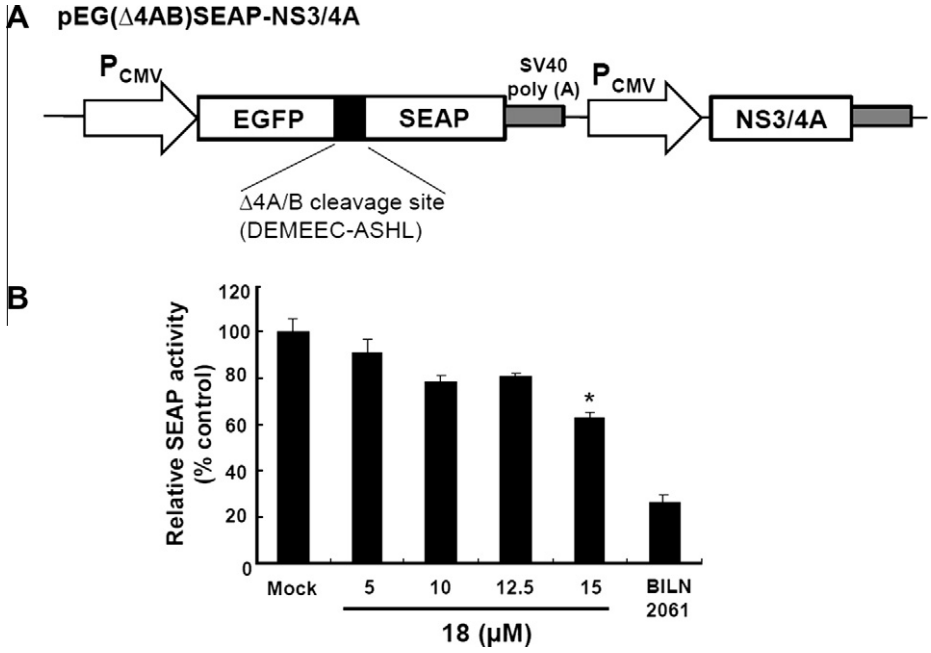


Figure 3. Cell-based HCV NS3/4A protease activity assay. (A) Schematic diagram of the HCV NS3/4A protease reporter construct, pEG(Δ4A/B)SEAP-NS3/4A. The construct contains the HCV *NS3/4A* protease gene and a dual-functional EG(Δ4A/B)SEAP reporter gene harboring a cleavage site of NS3/4A protease (designed Δ4A/B) inserted between the *egfp* and the *seap* genes. Both genes are controlled by the human cytomegalovirus immediate-early (CMV IE) promoter. (B) Transient reporter assay of NS3/4A protease. Huh-7 cells were transfected with 0.5 μg of pEG(Δ4AB)SEAP-NS3/4A plasmid for 6 h, and then were treated with compound **18** at concentration of 5, 10, 12.5 and 15 μM, in which treatment of 10 nM BILN 2061, a HCV protease inhibitor,[40] and 0.1% DMSO (mock) served as positive and negative control, respectively. After three days, the culture media were harvested to measure the SEAP activity. The efficacy of compound 18 against NS3/4A protease was reflected by the reductive percentage of SEAP activity compared with the mock control. The results are expressed as the means ± standard deviations (error bars) of triplicate experiments. $^{*}P < 0.05$.

subjected to western blot analysis with anti-HCV NS5B antibody, in which the level of GAPDH served as a loading control. The synthesis of HCV NS5B proteins was inhibited by compound **18** in a dose-dependent manner, confirming that compound **18** was a potential lead with anti-HCV activity (Fig. 2B). As the results showed, the inhibition of HCV replication of compound **18** is similar to the literature compound **4**.[20] This could be explained by the presence of the same core structure of quinoline ring. Comparable both EC_{50} and CC_{50} activities were observed by the peripheral substitution on the phenyl ring that plays an important role in the SI value of anti-HCV activity. Replacement of the electron-donating group and weak electron-withdrawing group on the phenyl ring showed the lower SI value (<1.1). On the contrary, introduction of strong electron-withdrawing group on the phenyl ring showed the higher SI value (2.4–10). Based on the structure of anilinoquinolines, we found that the features for optimum anti-HCV activities are: (1) substituent on the phenyl ring should be a strong electron-withdrawing group; (2) substituent at C-3 on phenyl ring is crucial in which the potency decreased in an order of **20** > **19** > **18**. To further analyze the effect of compound **18** on viral targets, such as internal ribosome entry site (IRES), NS3/4A protease activity, or NS5B polymerase activity, several reporter-based assays were performed.[37–39] The results indicated that compound **18** possessed anti-NS3/4A protease activity (Fig. 3B). In contrast, there no significant inhibition on IRES-mediated translation and NS5B polymerase activity (data not shown). However, we observed that the inhibitory effect of compound **18** on NS3/4A protease (~40%) is not consistent with that on HCV RNA level (~80%) at concentration of 15 μM, suggesting that there were multiple targets influenced by compound **18** for enhancement of anti-HCV activity.

We have synthesized and evaluated the anti-HCV activities of anilinoquinoline derivatives. Results indicated that introduction of a strong electron-withdrawing substituent such as NO_2 on the 2-anilinoquinolie pharmacophore is crucial for the suppression of HCV replication. Among them, 2-(3′-nitroanilino)quinoline (**18**) exhibited anti-HCV activity with an EC_{50} value of 7 μM and an SI value of 10. Inhibition of HCV NS3/4A protease may be partly responsible for the anti-HCV properties of compound **18**. This study discovered a new lead molecule to design more potent anti-HCV agents. Further structural modification of compound **18** as an anti-HCV candidate is currently in progress.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.121.

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