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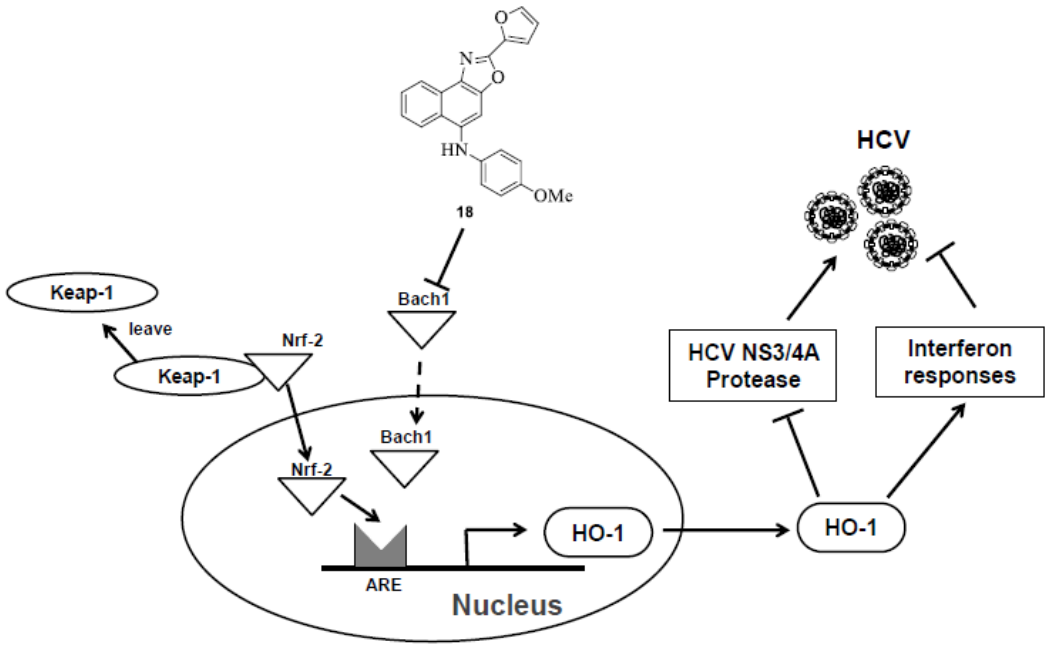
Graphical abstract



Discovery of naphtho[1,2-*d*]oxazole derivatives as potential anti-HCV agents through inducing heme oxygenase-1 expression

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Abstract

A number of naphtho[1,2-*d*]oxazole derivatives were synthesized and evaluated for their anti-HCV virus activity. Among them, compound **18** was the most active, exhibited approximately 21-folds more active anti-HCV activity (IC_{50} of 0.63 μM) than that of ribavirin (IC_{50} = 13.16 μM). Compound **18** was less cytotoxic than ribavirin, and the selective index (SI) of **18** is approximately 28-folds higher than that of ribavirin (229.10 *v.s.* 8.08). By using heme oxygenase-1 (HO-1) promoter-based assay and western blotting, compound **18** could induce HO-1 promoter activity, and protein expression. The antiviral effect of compound **18** was attenuated by HO-1 specific inhibitor SnPP treatment, which indicated that compound **18** suppressed HCV replication through inducing HO-1 expression. We further found that compound **18** reduced bach1 expression resulting in increasing the activity of Nrf-2 binding element. Moreover, the induction of HO-1 by compound **18** reduced HCV NS3/4A protease activity and induced the antiviral interferon responses. Therefore, compound **18** can be considered as a supplemental antiviral agent or a lead compound for further developing more effective agents against HCV replication.

Keywords: Naphtho[1,2-*d*]oxazole, Ribavirin, NS3/4A protease, Anti-HCV activity

1. Introduction

The Hepatitis C virus (HCV) belongs to the family Flaviviridae and is closely related to the West Nile virus (WNV), Yellow Fever virus (YFV), Japanese Encephalitis Virus (JEV), and Dengue virus (DENV) which contains a single-positive strand RNA genome [1,2]. HCV causes both acute and chronic infection, and eighty percent of HCV infection results in chronic liver disease, such as cirrhosis and hepatocellular carcinoma (HCC) [3-6]. Annually, approximately 3 million people are newly infected by HCV, and 350,000 to 500,000 people die from HCV relative liver disease [7]. Unfortunately, the development of HCV vaccine is impeded by viral genetic heterogeneity leading to immune evasion and the lake of a relevant animal model. The standard of care (SOC) for HCV patients is a combination treatment of pegylated interferon and synthetic guanosine ribavirin, but the sustained viral response rate is dependent on the HCV genotype, being successfully in 45-80%, and the severe adverse effect occurs commonly in a large number of patients [8,9]. Directly acting antiviral agent (DAAs) have been intensively developed and licensed in recent years, particularly targeting the viral NS3/4A protease, the NS5A phosphoprotein, and the NS5B polymerase, but the limitations of DAAs are the high cost, drug resistance and heavy pill load [10,11]. Discovering of therapeutic agents targeting host factors may help develop pan-genotype and less side effect antiviral strategy against HCV [12]. Therefore, the development of anti-HCV supplemental agents with higher effective and more affordable side effect profiles are still required to be discovered.

The heme oxygenase (HO-1), a heme-degrading enzyme, exhibits anti-inflammatory and antioxidant effect in vitro and in vivo [13]. Degradation of heme by HO-1 results in the production of biliverdin, ferrous iron, and carbon monoxide (CO), which are regarded as a potential protectant against liver injury [14]. HO-1 induction or overexpression has been demonstrated to reduce several virus replication, including HCV [15], DENV [16], hepatitis B virus (HBV) [17], and human immunodeficiency virus (HIV) [18]. In addition, the HO-1 product biliverdin, but not CO and ferrous iron, interferes with HCV replication by increasing endogenous anti-viral interferon

response and might directly inhibit the activity of HCV protease NS3/4A [19]. Consequently, the development of HO-1-inducible agents may offer a therapeutic strategy for HCV patients by simultaneously targeting both host and viral factors.

Previous study revealed that the most induction of HO-1 on the transcriptional level was regulated by kelch-like ECH-associated protein 1 (keap1)/NF-E2-related factor 2 (Nrf2) signaling pathway [20]. In the normal condition, keap1 protein is bound to the Nrf2 transcriptional factor, which results in Nrf2 protein degradation [20]. Upon the oxidative stress, the Nrf2 is released from keap1 and then translocated to the nucleus where it binds to antioxidant response element (ARE) resulting in transcriptional activation of HO-1 and other antioxidant enzymes [21]. In the absence of oxidative stress, the transcription factor *B*RCA1 *a*ssociated *C*-terminal *h*elicase 1 (Bach1), as a transcription inhibitor, forms heterodimers with the leucine zipper subfamily of small Maf proteins and binds to the Maf-recognition element (MARE) in the HO-1 promoter region [22]. The HO-1 expression is higher in the *bach1*-deficient mice compared with control mice, which indicated that bach1 is a repressor of HO-1 gene [23].

Many efforts had been devoted to the discovery of novel anti-HCV agents for the past few years [24-30]. In order to discover novel drug candidates, we have synthesized certain anilinoquinoline, anilinobenzothiazole, and anilinocoumarin derivatives and evaluated for their anti-HCV activities. Our results indicated that compound **1** inhibited the growth of HCV with an EC_{50} value of 7.0 μM [31]. Compound **2** inhibited HCV RNA-dependent RNA polymerase (RdRp) activity and HCV RNA replication (EC_{50} = 8 μM) [32]. Compound **3** exhibited strong anti-HCV activity at protein and RNA levels at non-toxic concentrations, with an EC_{50} value of 12 μM [33]. We also have discovered that compounds **4** and **5** inhibit the DENV2 RNA expression in Huh-7-DV-Fluc cells with potencies approximately equal to that of ribavirin [34]. In continuation of our efforts to discover potential antiviral agents, we demonstrate herein the synthesis of naphtho[1,2-*d*]oxazole derivatives (target compounds, *Figure* 1) and their evaluations related to the inhibition of HCV/NS5B by inducing HO-1 expression.

< **Insert Figure 1 here** >

2. Chemistry

Treatment of sodium 1,2-naphthoquinone-4-sulfonate (**6**) with 4-chloroaniline afforded 4-(4-chlorophenylamino)naphthalene-1,2-dione (**8)** in 74% yield. Preparation of compounds **7** and **9** were previously described [35]. The reaction of compounds **7** - **9** respectively with *p*-substituted benzaldehyde gave their corresponding naphtho[1,2-*d*]oxazole derivatives **10** – **12** in 55–75% yield. Methylation of compounds **10** – **12** with iodomethane afforded **13** - **15** as described in *Scheme 1*.

Under similar reaction conditions, compounds **7** – **9** were reacted with furaldehyde to give their corresponding naphtho[1,2-*d*]oxazole derivatives **16** – **18** in 58–74% yield. Methylation of compounds **16** – **18** with iodomethane afforded compounds **19** - **21** as described in *Scheme 2*.

< **Insert Scheme 1,2 here** >

3. Biological Results and Discussion

3.1. *Anti-HCV activities and Cytotoxicities*

The anti-HCV and cytotoxicities of naphtho[1,2-*d*]oxazole derivatives are summarized in *Table* 1. Ava-5 cells were treated with compounds **10** – **21** or the positive ribavirin respectively at a concentration of 5 or 20 μM for 3 days. HCV RNA levels were then determined by RT-qPCR analysis. The cell cytotoxicity was determined by XTT assay in the Ava-5 cells treatment with 20 and 200 μM of pyrazolylquinoline derivatives for 3 days. Compounds which exhibited > 50% inhibition of HCV at a concentration of 20 μM were considered as active. Results from *Table 1* indicated *N*,2-diphenylnaphtho[1,2-*d*]oxazol-5-amine (**10a**) and most of its substituted phenyl derivatives **10b**, **10c**, and **11** – **15** were inactive with the exception of compounds **11b** and **12a** which exhibited 55% and 87% inhibition of HCV at a concentration of 20 μM. 2-(Furan-2-yl)-*N*-phenylnaphtho[1,2-*d*]oxazol-5-amine (**16**) and its 4-chlorophenyl derivatives **17** were inactive. However, its 4-methoxyphenyl derivative **18** demonstrated a significant anti-HCV

activity, with a 95% inhibition of HCV. Further *N*-methylation of compound **18** reduced anti-HCV activity, in which compound **21** was inactive. The concentration that inhibited 50% DENV replication (IC_{50}), the concentration that inhibited 50% cell growth (CC_{50}), and the selective index (SI : CC_{50}/IC_{50}) of compound **11b**, **12a**, and **18** were determined with ribavirin as a positive control. Results from *Table* 2 indicated that compound **11b** was less active than ribavirin while compound **12a** was more active. Among them, compound **18** was the most active, exhibited approximately 21-folds more active anti-HCV activity (IC_{50} of 0.63 μM) than that of ribavirin (IC_{50} = 13.16 μM). In addition, compound **18** was less cytotoxic than ribavirin. The selective index (SI) of **18** is approximately 28-folds higher than that of ribavirin (229.10 *v.s.* 8.08).

< **Insert Table 1,2 here** >

3.2. *Compound **18** reduced HCV protein synthesis and RNA replication*

To further confirm the anti-HCV effect of compound **18**, we treated compound **18** at indicated concentrations in Ava5 cells for 3 days. The western blotting and RT-qPCR were performed to determine the HCV replication, and the results showed that compound **18** dose-dependently reduced HCV protein synthesis and RNA replication without cell cytotoxicity in HCV replicon cells (Fig. 2A and 2B). Compound **18** exhibited an IC_{50} value of 0.82 μM in reducing HCV RNA replication. We then further used the HCV JFH-1 infectious assay to confirm the inhibition effect of compound **18** on HCV replication under the same condition described above, and the result consistently showed that compound dose-dependently reduced HCV replication (Fig. 2c).

< **Insert figure 2 here** >

3.3. *Compound **18** reduced HCV replication through inducing HO-1 expression*

To determine whether compound **18** have impact on HO-1 gene expression in Ava5 cells, we first analyzed the level of HO-1 gene transcription in **18**-treated cells using HO-1 promoter-based luciferase reporter assay. Ava5 cell were transfected with pHO-1-Luc, a luciferase gene driven by HO-1 promoter, reporter plasmid and then were treated with compound **18** at indicated concentrations for 3 days. The result indicated that compound **18** significant induced HO-1

promoter activity in a concentration-dependent manner, compared with the DMSO-treated cells (Fig. 3A). Next, we performed western blotting to determine the HO-1 protein expression in Ava5 cells. As expected, compound **18** dose-dependently induced HO-1 protein level in Ava5 cells (Fig. 3B). To confirm the elevated HO-1 levels by compound **18** contributed to the anti-HCV replication, we used the specific inhibitor of HO-1 SnPP for evaluating the inhibition effect of compound **18** on HCV replication. Ava5 cells were co-incubated with 1 μM compound **18** and increasing concentration of SnPP (0-5 μM) for 3 days. As shown in Fig. 4A, SnPP attenuated the inhibition effect of compound **18** on HCV replication (lane 2-4), compared with SnPP-untreated cells (lane 2). Consistently, the RT-qPCR showed the similar result that SnPP restored the HCV RNA level under the same conditions described above (Fig. 4B).

< **Insert figure 3,4 here** >

3.4. *Anti-HCV activity of compound **18** is correlated with the down-regulation of bach-1 expression*

To investigate the detailed mechanism of compound **18**-induced HO-1 expression against HCV replication, we analyzed whether Nrf2, one of major transcription factors for activation of HO-1 expression, involved in anti-HCV activity. We treated compound **18** to Aca5 cells at indicated concentration. Results of Western blotting revealed that compound **18** did not affect Nrf2 and Keap-1 expression but dose-dependently reduced bach-1 expression (Fig. 5A). Furthermore, nuclear bach-1 protein level was also reduced in a dose-dependent manner in compound 18-treated Ava5 cells (Fig. 5B). To confirm the induction effect of HO-1 by compound **18** through reducing bach-1 expression leading to increasing Nrf2-mediated ARE activation for HO-1 expression, we transfected p2xARE-Luc, a reporter plasmid with ARE-driven firefly luciferase, into Ava5 cells and Huh-7 cells, followed by treatment of compound **18** at indicated concentrations. The result of luciferase activity showed that compound **18** dose-dependently increased activity of Nrf2 binding region, compared with Ava5 cells without compound **18** treatment (Fig. 5c).

< **Insert figure 5 here** >

3.5. *Compound **18** reduced HCV NS3/4A protease activity and activated antiviral IFN response*

To investigate whether HO-1-mediated reduction effect on HCV NS3/4A protease activity involved in the anti-viral effect of compound **18**, we performed a cell-based reporter assay by co-transfecting pEG(DE4AB)SEAP reporeter plasmid and pNS3/4A, a NS3/4A protein expression plasmid, into Huh-7 cells with compound **18** treatment at indicated concentration. The reporter plasmid was illustrated in Figure 6A, and the secreted SEAP activity in the cell culture medium displays NS3/4A protease activity. Results of SEAP activity indicated that compound **18** dose-dependently reduced NS3/4A protease activity (Fig. 6B). To further investigate whether the induction effect of compound **18** on HO-1 was able to activate cellular anti-viral interferon (IFN) response, we transfected a pISRE-luc reporter plasmid, an IFN-stimulated response element (ISRE)-driven firefly luciferase plasmid, and then incubated with compound **18** at increasing concentrations. As shown in Figure 7A, results of luciferase activity showed compound **18** significantly increased ISRE promoter activity in a dose-dependent manner. We further determined the ISRE-mediated IFN expression in the compound **18**-treated Ava5 cells by RT-qPCR. Similar results indicated that compound **18** dose-dependently increased IFN-α-2, -α-5 and -α-17 mRNA expression, compared with DMSO-treated Ava5 cells (Fig. 7B). The previous study revealed that activation of type I interferon could facilitate the expression of IFN-stimulated genes (ISGs) which are important regulators involved in immunomodulatory and antiviral responses against to viral infection, including HCV and hepatitis B virus [36]. To confirm whether compound **18** stimulated the expression of antiviral ISGs, including 2′-5′-oligoadenylate synthetase 1 (OAS1), OAS3, and protein kinase PKR, were determined by RT-qPCR. As shown in Figure 7C to E, compound **18** dose-dependently increased ISG expression at 1.0 and 2.5 μM.

< **Insert figure 6,7 here** >

4. Conclusion

In the present study, we revealed that compound **18**, 4-methoxyphenyl derivative of 2-(furan-2-yl)-*N*-phenylnaphtho[1,2-*d*]oxazol-5-amine, was the most effective anti-HCV agents among the naphtho[1,2-*d*]oxazole derivatives and exhibited IC_{50} values of 0.63 μM, compared with

the reference drug ribavirin (IC_{50} = 13 μM). The selective index (SI) of **18** is approximately 28-folds higher than that of ribavirin (229.10 *v.s.* 8.08) in Ava5 cells. We further determined the anti-viral mechanism of compound **18**, and our data exhibited that compound **18** strongly up-regulated HO-1 promoter activity and protein expression (Figs. 2 and 3). The anti-viral activity of compound **18** was attenuated by SnPP treatment (Fig. 4), which suggested that compound **18** reduced HCV replication through inducing HO-1 expression. The pathway of Nrf2-mediated HO-1 expression was investigated following exposure to compound **18**, and we found that compound 18 did not affect the Nrf2 and Keap1 expression but significantly reduced transcriptional repressor bach1 expression (Fig. 5). In addition, our results revealed that HO-1-mediated reduction effect on HCV NS3/4A protease activity and enhancement effect on the antiviral IFN response involved in antiviral activity of compound **18** (Figs. 6 and 7). Taken together, compound **18** reduced HCV replication through reducing bach1 expression resulting in HO-1 induction (Fig. 8). In addition, compound **18** is easy to be synthesized, which suggested that it can be a possible lead for further development of novel drug candidates against HCV replication.

< **Insert figure 8 here** >

5. Experimental

5.1. General

Melting points were determined on an Electrothermal IA9100 melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H and ^{13}C) spectra were recorded on a Varian Gemini 200 spectrometer or Varian-Unity-400 spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane (TMS) as an internal standard. Thin-layer chromatography was performed on silica gel 60 F-254 plates purchased from E. Merck and Co.. Mass spectra were recorded on Bruker APEX II (ESI) mass spectrometer. The elemental analyses were performed in the Instrument Center of National Science Council at National Cheng-Kung University and National Taiwan University using Heraeus CHN-O Rapid EA, and all values are within ± 0.4% of the theoretical compositions.

*5.2.1. 4-[(4-Chlorophenyl)amino]naphthalene-1,2-dione (**8**).* Compound **8** was prepared from 1,2-naphthoquinone-4-sulfonic acid sodium salt (**6**) and 4-chloroaniline according to literature conditions [35]. Yield 74% as a red solid. Mp. 275-276 ^{o}C (Dec). ^{1}H NMR (400 MHz, DMSO-d_6): 5.91 (br s, 1H, 3-H), 7.21–7.38 (m, 2H, Ar-H), 7.54 (m, 2H, Ar-H), 7.73–7.77 (m, 1H, 7-H), 7.85–7.89 (m, 1H, 6-H), 8.06 (d, 1H, J = 7.6 Hz, 5-H), 8.31 (d, 1H, J = 7.2 Hz, 8-H), 9.80 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): 100.65, 103.72, 115.23, 121.99, 123.94, 127.59 (2C), 128.33, 129.52 (2C), 133.53, 134.47, 149.38, 154.19, 176.17, 180.93. ESIMS $[M+H]^+$: 284. Anal. calcd for $C_{16}H_{10}ClNO_2$: C 67.74, H 3.55, N 4.94; found: C 67.65, H 3.42, N 5.05.

5.3. General procedure for the preparation of naphtho[1,2-d]oxazole compounds **10a-12c** and **16-18**.

To a suspension of **7, 8** or **9** (2.0 mmol) in acetic acid (15 mL) was added ammonium acetate (3.78 g, 49.0 mmol) and an appropriate benzaldehyde or furaldehyde (3.0 mmol). The reaction mixture was heated at 150^{o}C for 2.5 h (TLC monitoring). The solvent was removed in vacuo and the residue suspended in H_2O (20 mL). The crude product was purified by flash chromatography on silica gel and recrystallized with MeOH to afford the naphtho[1,2-*d*]oxazole products.

*5.3.1. N,2-Diphenylnaphtho[1,2-d]oxazol-5-amine (**10a**).* Yield 62% as a yellow solid. Mp. 158.0-159.0 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 6.02 (br s, 1H, NH), 6.96-7.00 (m, 1H, Ar-H), 7.08 (d, 2H, *J* = 7.6 Hz, Ar-H), 7.29-7.33 (m, 2H, Ar-H), 7.48-7.71 (m, 6H, Ar-H), 8.12 (d, 1H, *J* = 8.8 Hz, Ar-H), 8.27 (m, 2H, Ar-H), 8.61 (d, 1H, *J* = 8.0 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 100.35, 118.09, 121.22, 122.57, 122.91, 125.25, 125.65, 127.00 (2C), 127.39, 127.54, 128.70 (2C), 128.87 (2C), 129.54 (2C), 130.72, 133.17, 137.99, 144.13, 148.35, 161.45. ESIMS $[M+H]^+$: 337. Anal. calcd for $C_{23}H_{16}N_2O \cdot 0.1\ H_2O$: C 81.67, H 4.84, N 8.28; found: C 81.61, H 4.85, N 8.14.

*5.3.2. 2-(4-Chlorophenyl)-N-phenylnaphtho[1,2-d]oxazol-5-amine (**10b**).* Yield 71% as a yellow solid. Mp. 199-200 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 6.01 (br s, 1H, NH), 6.80-7.02 (m, 1H, Ar-H), 7.08 (m, 2H, Ar-H), 7.30-7.35 (m, 2H, Ar-H), 7.46-7.58 (m, 4H, Ar-H), 7.67-7.71 (m, 1H, Ar-H), 8.11 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.27 (m, 2H, Ar-H), 8.57 (d, 1H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (100

MHz, $CDCl_3$): 99.79, 118.28, 121.41, 122.53 (2C), 122.83, 125.34, 125.52, 126.04, 126.92, 127.48, 128.16 (2C), 129.18 (2C), 129.56 (2C), 132.99, 136.75, 138.32, 143.89, 148.45, 160.39. ESIMS $[M+H]^+$: 371. Anal. calcd for $C_{23}H_{15}ClN_2O$: C 74.49, H 4.08, N 7.55; found: C 74.14, H 3.93, N 7.57.

*5.3.3. 2-(4-methoxyphenyl)-N-phenylnaphtho[1,2-d]oxazol-5-amine (**10c**).* Yield 68% as a yellow solid. Mp. 184-185 ^oC. ^1H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OCH_3), 6.97-7.07 (m, 5H, Ar-H), 7.28-7.32 (m, 2H, Ar-H), 7.48.-7.52 (m, 2H, Ar-H), 7.64-7.68 (m, 1H, Ar-H), 8.10 (d, 1H, I = 8.8 Hz, Ar-H), 8.23 (m, 2H, Ar-H), 8.61 (m, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.44, 100.54, 114.37 (2C), 117.90, 119.71, 121.07, 122.66 (2C), 122.98, 125.22, 125.72, 127.33, 128.92 (2C), 129.51 (2C), 131.99, 132.46, 137.62, 144.26, 147.96, 161.59, 161.95. ESIMS $[M+H]^+$: 367. Anal. calcd for $C_{24}H_{18}N_2O_2$: C 78.67, H 4.95, N 7.65; found: C 78.93, H 4.92, N 7.72.

*5.3.4. N-(4-Chlorophenyl)-2-phenylnaphtho[1,2-d]oxazol-5-amine (**11a**).* Yield 63% as a yellow solid. Mp.: 174-175 ^oC. ^1H NMR (400 MHz, $CDCl_3$): 6.01 (br s, 1H, NH), 6.96 (d, 2H, *J* = 8.8 Hz, Ar-H), 7.22-7.26 (m, 2H, Ar-H), 7.51-7.55 (m, 5H, Ar-H), 7.66-7.70 (m, 1H, Ar-H), 8.07 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.27 (m, 2H, Ar-H), 8.61 (d, 1H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 101.32, 118.85, 122.65 (2C), 122.97, 125.42, 125.65, 125.84, 127.07 (2C), 127.33, 127.51, 128.92 (2C), 129.14, 129.45 (2C), 130.91, 133.49, 137.40, 143.07, 148.09, 161.66. ESIMS $[M+H]^+$: 371. Anal. calcd for $C_{23}H_{15}ClN_2O$: C 74.49, H 4.08, N 7.55; found: C 74.62, H 3.95, N 7.49.

*5.3.5. N,2-Bis(4-chlorophenyl)naphtho[1,2-d]oxazol-5-amine (**11b**).* Yield 56% as a yellow solid. Mp.: 206-207 ^oC. ^1H NMR (400 MHz, $CDCl_3$): 6.01 (br s, 1H, NH), 6.96-6.99 (m, 2H, Ar-H), 7.23-7.27 (m, 2H, Ar-H), 7.48-7.57 (m, 4H, Ar-H), 7.68-7.72 (m, 1H, Ar-H), 8.08 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.18-8.22 (m, 2H, Ar-H), 8.59 (d, 1H, *J* = 8.0 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 100.95, 119.03, 122.60 (2C), 122.91, 125.52, 125.78, 125.86, 125.97, 126.97, 127.61, 128.24 (2C), 129.23 (2C), 129.48 (2C), 133.61, 136.92, 137.65, 142.90, 148.24, 160.68. ESIMS $[M+H]^+$: 405. Anal. calcd for $C_{23}H_{14}Cl_2N_2O$: C 68.16, H 3.48, N 6.91; found: C 68.13, H 3.43, N 7.31.

5.3.6. *N-(4-Chlorophenyl)-2-(4-methoxyphenyl)naphtho[1,2-d]oxazol-5-amine (**11c**).* Yield 62% as a red solid. Mp.: 191-192 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.89 (s, 3H, OCH_3), 6.01 (br s, 1H, NH), 6.91-6.94 (m, 2H, Ar-H), 7.00-7.04 (m, 2H, Ar-H), 7.20-7.25 (m, 2H, Ar-H), 7.47-7.51 (m, 2H, Ar-H), 7.63-7.67 (m, 1H, Ar-H), 8.04 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.19-8.22 (m, 2H, Ar-H), 8.58 (d, 1H, *J* = 8.0 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.43, 101.82, 114.36 (2C), 118.53, 119.79, 122.73 (2C), 122.94, 125.30, 125.95, 127.34 , 128.88 (2C), 129.39 (2C), 129.79, 132.45, 133.44, 134.96, 136.80, 143.33, 147.73, 161.93. ESIMS $[M+H]^+$: 401. Anal. calcd for $C_{24}H_{17}ClN_2O_2$: C 71.91, H 4.27, N 6.99; found: C 71.55, H 4.24, N 7.26.

5.3.7. *N-(4-Methoxyphenyl)-2-phenylnaphtho[1,2-d]oxazol-5-amine (**12a**).* Yield 55% as a yellow solid. Mp.: 210-211 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.84 (s, 3H, OCH_3), 6.01 (br s, 1H, NH), 6.93-6.95 (m, 2H, Ar-H), 7.10-7.14 (m, 2H, Ar-H), 7.47-7.58 (m, 5H, Ar-H), 7.66-7.70 (m, 1H, Ar-H), 8.09 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.24-8.28 (m, 2H, Ar-H), 8.61-8.62 (m, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.62, 95.98, 115.00, 117.66, 121.82 (2C), 122.87, 123.04, 123.98, 125.04 , 126.91 (2C), 127.36, 128.86 (2C), 130.61 (2C), 131.09, 135.99, 140.78, 143.08, 148.82, 155.76, 160.69. ESIMS $[M+H]^+$: 367. Anal. calcd for $C_{24}H_{18}N_2O_2$: C 78.67, H 4.95, N 7.65 found: C 78.34, H 5.25, N 7.27.

5.3.8. *2-(4-Chlorophenyl)-N-(4-methoxyphenyl)naphtho[1,2-d]oxazol-5-amine (**12b**).* Yield 69% as a yellow solid. Mp.: 190-191 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.84 (s, 3H, OCH_3), 6.00 (br s, 1H, NH), 6.92-6.96 (m, 2H, Ar-H), 7.13-7.15 (m, 2H, Ar-H), 7.44-7.56 (m, 4H, Ar-H), 7.66-7.70 (m, 1H, Ar-H), 8.08 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.13-8.15 (m, 2H, Ar-H), 8.55 (d, 1H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.61, 95.63, 114.97 (2C), 121.79, 122.85, 122.97 (2C), 123.85, 125.04, 126.20, 126.92 , 127.39, 127.97 (2C), 128.39, 129.12 (2C), 129.23, 131.45, 136.43, 148.96, 155.78, 159.72. ESIMS $[M+H]^+$: 401. Anal. calcd for $C_{24}H_{17}ClN_2O_2$: C 71.91, H 4.27, N 6.99; found: C 71.54, H 4.04, N 6.91.

5.3.9. *N,2-Bis(4-methoxyphenyl)naphtho[1,2-d]oxazol-5-amine (**12c**).* Yield 75% as a yellow solid. Mp.: 186-187 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.83 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.91-6.93

(m, 2H, Ar-H), 6.99-7.18 (m, 5H, Ar-H), 7.52 (br s, 1H, N-H), 7.64-7.67 (m, 2H, Ar-H), 8.08 (d, 1H, J = 8.8 Hz, Ar-H), 8.20-8.21 (m, 2H, Ar-H), 8.61 (m, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 55.42, 55.60, 96.20, 114.34 (2C), 114.97 (2C), 119.65, 121.90 (2C), 122.56, 123.06, 123.97, 124.97, 127.29, 128.77 (2C), 130.49, 131.09, 136.09, 140.41, 148.44, 155.57, 160.86, 161.82. ESIMS $[M+H]^+$: 397. Anal. calcd for $C_{25}H_{20}N_2O_3$: C 75.74, H 5.08, N 7.07; found: C 75.37, H 5.08, N 7.19.

5.3.10. *2-(Furan-2-yl)-N-phenylnaphtho[1,2-d]oxazol-5-amine (**16**).* Yield 63% as a yellow solid. Mp.: 125-126 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 6.61 (dd, *J* = 3.2, 1.6 Hz, 1H, Fu-4-H), 6.97-7.00 (m, 1H, Ar-H), 7.07-7.09 (m, 2H, Ar-H), 7.22 (d, 1H, *J* = 3.2 Hz, Fu-3-H), 7.29-7.33 (m, 2H, Ar-H), 7.51-7.55 (m, 2H, Ar-H and N-H), 7.65-7.69 (m, 2H, Ar-H), 8.10 (d, 1H, *J* = 8.4 Hz, Ar-H), 8.60 (d, 1H, *J* = 8.4 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 99.67, 112.18, 112.85, 118.27, 121.41, 122.48 (2C), 122.96, 125.39, 125.57, 126.76, 127.51, 129.53 (2C), 132.32, 138.38, 143.86, 145.08, 147.81, 150.28, 153.76. ESIMS $[M+H]^+$: 327. Anal. calcd for $C_{21}H_{14}N_2O_2$: C 77.29, H 4.32, N 8.58; found: C 77.12, H 4.21, N 8.45.

5.3.10. *N-(4-Chlorophenyl)-2-(furan-2-yl)naphtho[1,2-d]oxazol-5-amine (**17**).* Yield 74% as a yellow solid. Mp.: 172-173 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 6.10 (br s, 1H, NH), 6.62 (dd, *J* = 3.2, 1.6 Hz, 1H, Fu-4-H), 6.94-6.98 (m, 2H, Ar-H), 7.22-7.26 (m, 3H, Ar-H), 7.53-7.57 (m, 2H, Ar-H), 7.67-7.71 (m, 2H, Ar-H), 8.07 (dd, 1H, *J* = 8.4, 0.8 Hz, Ar-H), 8.61 (d, 1H, *J* = 8.0 Hz, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$): 100.91, 112.20, 112.94, 118.97,121.36, 122.57, 123.01 (2C), 125.55, 125.85, 126.87, 127.61, 129.46 (2C), 133.14, 137.65, 142.88, 145.16, 147.61, 151.06, 154.08. ESIMS $[M+H]^+$: 361. Anal. calcd for $C_{21}H_{13}ClN_2O_2 \cdot 0.6\ H_2O$: C 67.87, H 3.85, N 7.54; found: C 67.56, H 3.75, N 7.54.

5.3.11. *2-(Furan-2-yl)-N-(4-methoxyphenyl)naphtho[1,2-d]oxazol-5-amine (**18**).* Yield 58% as a yellow solid. Mp.: 175-176 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.83 (s, 3H, OCH_3), 6.01 (br s, 1H, NH), 6.59 (dd, *J* = 3.2, 1.6 Hz, 1H, Fu-4-H), 6.92 (d, 2H, J = 8.4 Hz, Ar-H), 7.11-7.20 (m, 3H, Ar-H), 7.52-7.70 (m, 3H, Ar-H), 8.08 (d, 1H, J = 8.8 Hz, Ar-H), 8.59 (m, 1H, Ar-H). ^{13}C NMR

(100 MHz, $CDCl_3$): 55.61, 95.54, 112.09 (2C), 112.31, 114.96, 117.77, 120.81, 121.74 (2C), 123.01, 123.91, 125.12, 127.42, 130.79, 135.44, 140.99, 144.85, 148.19, 153.12, 155.81. ESIMS $[M+H]^+$: 357. Anal. calcd for $C_{22}H_{16}N_2O_3$: C 74.15, H 4.53, N 7.86; found: C 74.22, H 4.38, N 7.77.

5.4. General procedure for preparation of N-methyl-N,2-diphenylnaphtho[1,2-d]oxazole derivatives: ***13a-15c*** *and* ***19-21***.

To a stirred solution of *naphtho[1,2-d]oxazole* derivatives **10a-12c** or **16-18** (1.0 mmol) in dry DMF (20 mL) was added NaH (60% in oil, 0.50 g) at 0^oC and methyliodide (3 mmol) was added at rt for 10 min (TLC monitoring). The organic phase was washed with brine, dried over $MgSO_4$, filtered and concentrated under *vacuo*. Crude product was purified by flash chromatography on silica gel (MeOH/CH_2Cl_2 1/50) and crystallized from MeOH.

*5.4.1. N-Methyl-N,2-diphenylnaphtho[1,2-d]oxazol-5-amine (****13a****).* Yield 78% as a yellow solid. Mp. 124-125 ^oC. ^1H NMR (400 MHz, $CDCl_3$): 3.44 (s, 3H, NCH_3), 6.65-6.68 (m, 2H, Ar-H), 6.74-6.79 (m, 1H, Ar-H), 7.15-7.21 (m, 2H, Ar-H), 7.47-7.57 (m, 4H, Ar-H), 7.66-7.70 (m, 2H, Ar-H), 7.98 (d, 1H, *J* = 8.4 Hz, 9-H), 8.30-8.33 (m, 2H, Ar-H), 8.64-8.67 (m, 1H, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$): 40.54, 110.74, 113.84 (2C), 117.69, 122.83, 124.84, 125.84, 127.28 (2C), 127.32, 127.39, 127.42, 128.92 (2C), 129.00 (2C), 129.14, 131.10, 136.35, 143.80, 148.02, 150.08, 162.74. ESIMS $[M+H]^+$: 351. Anal. calcd for $C_{24}H_{18}N_2O$: C 82.26, H 5.18, N 7.99; found: C 81.96, H 5.38, N 7.72.

*5.4.2. 2-(4-Chlorophenyl)-N-methyl-N-phenylnaphtho[1,2-d]oxazol-5-amine (****13b****).* Yield 83% as a yellow solid. Mp: 150-151 ^oC. ^1H NMR (400 MHz, $CDCl_3$): 3.45 (s, 3H, NCH_3), 6.66-6.68 (m, 2H, Ar-H), 6.75-6.79 (m, 1H, Ar-H), 7.16-7.20 (m, 2H, Ar-H), 7.48-7.54 (m, 3H, Ar-H), 7.65 (s, 1H, 4-H), 7.67-7.71 (m, 1H, 7-H), 7.99 (d, 1H, *J* = 8.4 Hz, 9-H), 8.23-8.26 (m, 2H, Ar-H), 8.62 (d, 1H, *J* = 8.0 Hz, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$): 40.56, 110.64, 113.92 (2C), 117.81, 122.78, 124.90, 125.92, 125.96, 127.29, 127.51, 128.51 (2C), 129.02 (2C), 129.20, 129.28 (2C), 136.27, 137.27,

144.13, 148.09, 150.04, 161.73. ESIMS $[M+H]^+$: 385. Anal. calcd for $C_{24}H_{17}ClN_2O \cdot 0.4\ H_2O$: C 73.52, H 4.58, N 7.15; found: C 73.24, H 4.30, N 7.01.

*5.4.3. 2-(4-Methoxyphenyl)-N-methyl-N-phenylnaphtho[1,2-d]oxazol-5-amine (**13c**).* Yield 81% as a yellow solid. Mp. 129-130 ^oC. ^1H NMR (400 MHz, $CDCl_3$): 3.44 (s, 3H, NCH_3), 3.90 (s, 3H, OCH_3), 6.64-6.67 (m, 2H, Ar-H), 6.74-6.78 (m, 1H, Ar-H), 7.03-7.07 (m, 2H, Ar-H), 7.15-7.20 (m, 2H, Ar-H), 7.46-7.50 (m, 1H, 8-H), 7.64-7.69 (m, 2H, 4- and 7-H), 7.97 (d, 1H, *J* = 8.4 Hz, 9-H), 8.24-8.27 (m, 2H, Ar-H), 8.62-8.64 (m, 1H, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$): 40.51, 55.45, 110.77, 113.73 (2C), 114.37 (2C), 117.57, 120.06, 122.82, 124.79, 125.71, 127.17, 127.23, 128.98 (2C), 129.05 (2C), 129.09, 136.48, 143.20, 147.80, 150.12, 162.02, 162.95. ESIMS $[M+H]^+$: 381. Anal. calcd for $C_{25}H_{20}N_2O_2 \cdot 0.6\ H_2O$: C 76.75, H 5.46, N 7.16; found: C 76.67, H 5.15, N 7.37.

*5.4.4. N-(4-Chlorophenyl)-N-methyl-2-phenylnaphtho[1,2-d]oxazol-5-amine (**14a**).* Yield 75% as a white solid. Mp. 159-160 ^oC. ^1H NMR (400 MHz, $CDCl_3$): 3.43 (s, 3H, NCH_3), 6.56-6.58 (m, 2H, Ar-H), 7.10-7.12 (m, 2H, Ar-H), 7.49-7.57 (m, 4H, Ar-H), 7.65 (s, 1H, 4-H), 7.67-7.71 (m, 1H, 7-H), 7.92 (d, 1H, *J* = 8.8 Hz, 9-H), 8.32 (m, 2H, Ar-H), 8.65 (d, 1H, *J* = 8.0 Hz, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$): 40.67, 110.81, 114.85 (2C), 122.60, 122.95, 124.59, 125.31, 126.00, 127.33 (2C), 127.33, 127.51, 128.82 (2C), 128.87, 128.95 (2C), 131.20, 136.61, 143.17, 147.91, 148.67, 162.91. ESIMS $[M+H]^+$: 385. Anal. calcd for $C_{24}H_{17}ClN_2O \cdot 0.4\ H_2O$: C 73.52, H 4.58, N 7.15; found: C 73.35, H 4.47, N 7.03.

*5.4.5. N,2-bis(4-Chlorophenyl)-N-methylnaphtho[1,2-d]oxazol-5-amine (**14b**).* Yield 75% as a yellow solid. Mp. 213-214 ^oC. ^1H NMR (400 MHz, $CDCl_3$): 3.43 (s, 3H, NCH_3), 6.55-6.59 (m, 2H, Ar-H), 7.09-7.13 (m, 2H, Ar-H), 7.49-7.53 (m, 3H, Ar-H), 7.63 (s, 1H, 4-H), 7.68-7.71 (m, 1H, 7-H), 7.92 (d, 1H, *J* = 8.4 Hz, 9-H), 8.24-8.26 (m, 2H, Ar-H), 8.63 (d, 1H, *J* = 8.4 Hz, 6-H**)**. ^{13}C NMR (100 MHz, $CDCl_3$): 40.69, 110.69, 114.91 (2C), 122.69, 122.89, 124.64, 125.82, 126.11, 127.32, 127.63, 128.53 (2C), 128.84 (2C), 128.92, 129.30 (2C), 136.52, 137.37, 143.48, 147.97, 148.63, 161.90. ESIMS $[M+H]^+$: 419. Anal. calcd for $C_{24}H_{16}Cl_2N_2O \cdot 0.5\ H_2O$: C 67.59, H 3.97, N 6.57; found: C 67.34, H 3.75, N 6.84.

*5.4.6. N-(4-Chlorophenyl)-2-(4-methoxyphenyl)-N-methylnaphtho[1,2-d]oxazol-5-amine (**14c**).* Yield 76% as a yellow solid. Mp. 172-173 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.42 (s, 3H, NCH_3), 3.91 (s, 3H, OCH_3), 6.54-6.58 (m, 2H, Ar-H), 7.04-7.13 (m, 4H, Ar-H), 7.47-7.51 (m, 1H, 8-H), 7.63 (s, 1H, 4-H), 7.66-7.70 (m, 1H, 7-H), 7.90 (d, 1H, *J* = 8.4 Hz, 9-H), 8.25-8.28 (m, 2H, Ar-H), 8.64-8.66 (m, 1H, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$): 40.65, 55.47, 110.76, 114.43 (2C), 114.78 (2C), 119.85, 122.51, 122.98, 124.53, 125.90, 127.14, 127.38, 128.81 (2C), 128.84, 129.14 (2C), 136.52, 142.65, 147.67, 148.72, 162.16, 163.11. ESIMS $[M+H]^+$: 415. Anal. calcd for $C_{25}H_{19}ClN_2O_2$: C 72.37, H 4.62, N 6.75; found: C 72.38, H 4.76, N 6.87.

*5.4.7. N-(4-Methoxyphenyl)-N-methyl-2-phenylnaphtho[1,2-d]oxazol-5-amine (**15a**).* Yield 71% as a yellow solid. Mp. 120-121 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.41 (s, 3H, NCH_3), 3.75 (s, 3H, OCH_3), 6.67-6.71 (m, 2H, Ar-H), 6.76-6.79 (m, 2H, Ar-H), 7.46-7.57 (m, 4H, Ar-H), 7.60 (s, 1H, 4-H), 7.64-7.68 (m, 1H, 7-H), 8.05-8.07 (m, 1H, 9-H), 8.30-8.32 (m, 2H, Ar-H), 8.62-8.64 (m, 1H, 6-H**)**. ^{13}C NMR (100 MHz, $CDCl_3$): 41.47, 55.68, 109.21, 114.54 (2C), 116.48 (2C), 122.74, 123.04, 123.98, 125.08, 125.58, 127.21 (2C), 127.27, 127.48, 128.91 (2C), 130.99, 135.92, 144.87, 145.16, 148.14, 152.65, 162.42. ESIMS $[M+H]^+$: 381. Anal. calcd for $C_{25}H_{20}N_2O_2$: C 78.93, H 5.30, N 7.36; found: C 78.83, H 5.24, N 7.23.

*5.4.8. 2-(4-Chlorophenyl)-N-(4-methoxyphenyl)-N-methylnaphtho[1,2-d]oxazol-5-amine (**15b**).* Yield 78% as a yellow solid. Mp. 137-1338 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.41 (s, 3H, NCH_3), 3.75 (s, 3H, OCH_3), 6.68-6.78 (m, 4H, Ar-H), 7.46-7.53 (m, 3H, Ar-H), 7.58 (s, 1H, 4-H), 7.65-7.69 (m, 1H, 7-H), 8.06 (d, 1H, *J* = 8.4 Hz, 9-H), 8.22-8.26 (m, 2H, Ar-H), 8.59 (d, 1H, *J* = 8.4 Hz, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$): 41.52, 55.68, 109.01, 114.55 (2C), 116.63 (2C), 122.66, 125.15, 125.68, 126.01, 127.37, 128.42 (2C), 128.93, 129.25 (2C), 130.25, 135.57, 137.11, 144.84, 145.48, 148.22, 152.75, 161.41. ESIMS $[M+H]^+$: 415. Anal. calcd for $C_{25}H_{19}ClN_2O_2 \cdot 0.5\ H_2O$: C 70.84, H 4.76, N 6.61; found: C 70.53, H 4.62, N 6.72.

*5.4.9. N,2-Bis(4-methoxyphenyl)-N-methylnaphtho[1,2-d]oxazol-5-amine (**15c**).* Yield 75% as a yellow solid. Mp. 119-120 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.40 (s, 3H, NCH_3), 3.74 (s, 3H,

OCH_3), 3.90 (s, 3H, OCH_3), 6.66-6.69 (m, 2H, Ar-H), 6.75-6.78 (m, 2H, Ar-H), 7.03-7.06 (m, 2H, Ar-H), 7.44-7.49 (m, 1H, 8-H), 7.58 (s, 1H, 4-H), 7.63-7.67 (m, 1H, 7-H), 8.04 (d, 1H, *J* = 8.4 Hz, 9-H), 8.23-8.27 (m, 2H, Ar-H), 8.61-8.63 (m, 1H, 6-H**)**. ^{13}C NMR (100 MHz, $CDCl_3$): 41.38, 55.43, 55.68, 109.29, 114.36 (2C), 114.53 (2C), 116.26, 120.00, 122.75, 125.00, 125.48, 127.03, 127.14, 128.87, 128.99 (2C), 135.60, 144.59, 144.90, 147.86, 152.54, 161.98, 162.62. ESIMS $[M+H]^+$: 411. Anal. calcd for $C_{26}H_{22}N_2O_3$: C 76.08, H 5.40, N 6.82; found: C 76.04, H 5.39, N 6.78.

*5.4.10. 2-(Furan-2-yl)-N-methyl-N-phenylnaphtho[1,2-d]oxazol-5-amine (**19**).* Yield 71% as a gray solid. Mp. 126-127 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.44 (s, 3H, NCH_3), 6.63-6.68 (m, 3H, Ar-H), 6.75-6.79 (m, 1H, Ar-H), 7.16-7.20 (m, 2H, Ar-H), 7.27 (dd, 1H, *J* = 3.6, 0.8 Hz, Fu-3-H), 7.48-7.52 (m, 1H, 8r-H), 7.63 (s, 1H, 4-H), 7.63-7.70 (m, 2H, Ar-H), 8.05 (d, 1H, *J* = 8.4 Hz, 9-H), 8.64-8.67 (m, 1H, 6-H). ^{13}C-NMR (100 MHz, $CDCl_3$): 40.54, 110.52, 112.26, 113.50, 113.90 (2C), 117.79, 122.88, 124.85, 126.01, 127.17, 127.52, 129.01 (2C), 129.25, 135.81, 142.78, 144.10, 145.43, 147.44, 155.00, 155.04. ESIMS $[M+H]^+$: 341. Anal. calcd for $C_{22}H_{16}N_2O_2$: C 77.63, H 4.74, N 8.23; found: C 77.58, H 4.81, N 7.99.

*5.4.11. N-(4-Chlorophenyl)-2-(furan-2-yl)-N-methylnaphtho[1,2-d]oxazol-5-amine (**20**).* Yield 79% as a yellow solid. Mp. 200-201 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.42 (s, 3H, NCH_3), 6.54-6.58 (m, 2H, Ar-H), 6.64 (dd, 1H, *J* = 3.6, 2.0 Hz, Fu-4-H), 7.09-7.13 (m, 2H, Ar-H), 7.29 (dd, 1H, *J* = 3.6, 0.8 Hz, Fu-3-H), 7.49-7.53 (m, 1H, 8-H), 7.62 (s, 1H, 4-H), 7.67-7.71 (m, 2H, Ar-H), 7.91 (d, 1H, *J* = 8.4 Hz, 9-H), 8.66 (d, 1H, *J* = 8.0 Hz, 6-H). ^{13}C-NMR (100 MHz, $CDCl_3$): 40.68, 110.57, 112.29, 113.64, 114.90 (2C), 122.70, 123.00, 124.61, 126.17, 127.21, 127.64, 128.84 (2C), 128.90, 136.10, 142.73, 143.47, 145.51, 147.33, 148.60, 155.19. ESIMS $[M+H]^+$: 375. Anal. calcd for $C_{22}H_{15}ClN_2O_2 \cdot 0.3\ H_2O$: C 69.49, H 4.14, N 7.37; found: C 69.28, H 3.92, N 7.27.

*5.4.12. 2-(Furan-2-yl)-N-(4-methoxyphenyl)-N-methylnaphtho[1,2-d]oxazol-5-amine (**21**).* Yield 76% as a yellow solid. Mp. 146-147 ^{o}C. ^{1}H NMR (400 MHz, $CDCl_3$): 3.40 (s, 3H, NCH_3), 3.75 (s, 3H, OCH_3), 6.63 (dd, 1H, *J* = 3.6, 1.6 Hz, Fu-4-H), 6.66-6.79 (m, 4H, Ar-H), 7.26 (dd, 1H, *J* = 3.6, 0.8 Hz, Fu-3-H), 7.46-7.50 (m, 1H, 8-H), 7.57 (s, 1H, 4-H), 7.64-7.67 (m, 1H, 7-H), 7.68 (dd, 1H, *J*

= 1.6, 0.8 Hz, Fu-5-H), 8.05 (d, 1H, *J* = 8.8 Hz, 9-H), 8.62-8.64 (m, 1H, 6-H). ^{13}C NMR (100 MHz, $CDCl_3$): 41.51, 55.67, 108.89, 112.22, 113.25, 114.53 (2C), 116.61 (2C), 122.78, 125.10, 125.73, 127.10, 127.39, 128.96, 135.08, 142.86, 144.78, 145.31, 145.44, 147.57, 152.73, 154.74. ESIMS $[M+H]^+$: 371. Anal. calcd for $C_{23}H_{18}N_2O_3$: C 74.58, H 4.90, N 7.56; found: C 74.49, H 4.85, N 7.51.

5.5. Cytotoxicity and antiviral activity assays

5.5.1. Compounds

Compounds were dissolved in DMSO at 10 mM and then diluted in culture medium.

5.5.2. Cells culture and reagents

Ava5 cells [37], human hepatoma cells (Huh-7) harbaoring HCV replicon genome, were cultured in DMEM culture medium with 10% heat-inactivated fetal bovine serum, 1% antibiotic–antimycotic, 1% non-essential amino acids and 1 $mg \cdot mL^{-1}$ G418 and . Huh-7 cells were maintained in DMEM with 10% heat-inactivated fetal bovine serum, 1% antibiotic-antimycotic, and 1% non-essential amino acids and were incubated at 37 °C with a 5% CO_2 supplement. An HO-1-specific inhibitor [tin protoporphyrin IX dichloride (SnPP)] was purchased from Sigma (St. Louis, MO, USA).

5.5.3. Cytotoxicity assays

For cytotoxicity tests, run in parallel with antiviral assays, plates at an initial density of (5×10^3 cells/well) were treated with or without serial dilutions of test compounds. Cell viability was determined after 72 h at 37 ^oC in a humidified CO_2 (5%) atmosphere by the (2,3-bis [2-methyloxy-4-nitro-5-sulfophenyl]-2*H*-tetrazolium-5-carboxanilide) (XTT) method [38].

5.5.4. Tansfection and luciferase activity assay

Ava5 cells were transfected with the HO-1 promoter-driven luciferase plasmid, pHO-1-Luc, using the T-pro^{TM} transfection reagent (Ji-Feng Biotechnology Co., Ltd., Taipei, Taiwan) according to the manufacturer's instructions. The transfected cells were treated with compound **18** at various concentrations for 3 days. Each transfection complex contains 0.1 μg pCMV-SEAP, a secreted alkaline phosphatase (SEAP) expression vector, serving as a transfection control for normalization

luciferase activity. The luciferase activity assay was performed using the Bright-Glo Luciferase assay system (Promega) according to the manufacturer's instructions.

5.5.5. Immunoblot analysis

Ava5 cells were seeded in 24-well plates at a density of 5×10^4 cells per well for over-night and treated with indicated reagent at proper concentrations for 3 days. Cells were washed twice with cold PBS and lysed by RIPA lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 2 mM EDTA, 1 mM EGTA, 1 mM $NaVO_3$, 10 mM NaF, 1 mM DTT, 1 mM PMSF, 25 μg/mL aprotinin, and 25 μg/mL leupeptin) and stored at −20 °C. The protein concentration was determined by the Bradford method. 10 μg protein were separated by 10% SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked with 5% non-fat dried milk and incubated with anti-NS5B, anti-GAPDH and anti-HO-1 antibodies, followed by 30 min with secondary antibodies in 5% milk containing Tris-buffered saline (TBS) and 0.5% Tween. The blotting signal was developed using an ECL detection kit (PerkinElmer, CT, USA) and was counted by the software Quantity One (Bio-Rad, CA, USA) [39].

5.5.6. Real-time quantitative PCR (RT-qPCR) assay

Total cellular RNA were extracted by a Total RNA Miniprep Purification kit (GMbiolab, Taiwan) according to the manufacturer's instructions and were transcribed to cDNA by M-MLV reverse transcriptase (Promega, USA) with HCV 3'UTR (5'-acttgatctgcagagaggcc-3') or oligo dT primer. The mRNA expression of target gene were determined by quantitative real-time RT-PCR with specific primers as previous study described [15]. The CT value of each sample was determined by the ABI Step One Real-Time PCR-System. The PCR primers were as follows: GAPDH, 5'-gtcttcaccaccatggagaa-3' (forward), and 5'-atggcatggactgtggtcat-3' (reverse); NS5B, 5'-ggaaaccaagctgcccatca-3' (forward), and 5'-cctccacggatagaagttta-3' (reverse). Each relative mRNA levels were obtained by normalization of the endogenous cellular *gapdh* gene.

5.5.7. Preparation of HCVcc stock and viral infection assay

Huh-7 cells stably expressing pEF/JFH1-Rz/N plasmid were cultured to produce cell culture-drived infectious HCV (HCVcc), and the condition medium were collected to harvest HCV particles [40]. Fivity percent cell culture infective dose of harvested vrial particle was determined by immunofluorescence assay with the specific anti-Core antibodies (Abcam). For the antiviral activity assay, the Huh-7 cells were seeded into a 24 well plate with the density of 5×10^4 cells per well and infected with HCVcc at a multiplicity of infection of 0.1 for 6 hr. The infected cells were treated with various concentrations of compound **18** for 3 days. The RT-qPCR was used to analyze the anti-HCV activity of compound **18**.

5.5.8. Statistical analysis

All data were presented as mean ± SD of three independent experiments with triplicate sample. Statistical analysis was calculated by Student's *t*-test, and the significant difference was considered if *P<0.05.

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Figure captions:

Figure 1. Structures of 3'-nitrophenylaminoquinoline (**1**), 2-(4-nitroanilino)-6-methylbenzothiazole (**2**), 3-(3',4',5'-trimethoxyanilin-1'-yl)methylaminocoumarin (**3**), 2-(hydroxyphenylmethyl)-3-(4-methoxyphenyl)quinoline (**4**), 2-(4-hydroxybenzoyl)-3-(4-hydroxyphenyl)quinoline (**5**), and target compounds.

Figure 2. Compound 18 dose-dependently reduced HCV replication. Compound **18** reduced HCV (A) protein synthesis and (B) RNA replication in Ava5 cells. Ava5 cells were treated with compound **18** at indicated concentrations for 3 days. Cell lysates and cellular mRNA were subjected to Western blotting and RT-qPCR. Relative HCV RNA were normalized by cellular *gapdh* mRNA levels. Cell variability was determined in compound **18**-treated Ava5 cells at indicated concentrations for 3 days. (C) Compound **18** inhibited HCV JFH-1 replication in Huh7.5 cells in a dose-dependently manner. Huh7.5 cells were infected with HCV JFH-1 at an MOI of 1, and the infected cells were treated with compound **18** for 3 days. RT-qPCR was performed to determined relative HCV RNA level. Data was presented as the mean ± SD of three independent experiments; $*P < 0.05$.

Figure 3. Compound 18 induced HO-1 expression in Ava5 cells. Compound **18** induced (A) HO-1 promoter activity and (B) HO-1 protein expression. Ava5 cells were transfected with pHO-1-Luc at 0.5 μg, and the transfected cells were treated with compound **18** for 3 days. The cell lysate was subjected to luciferase activity assay. Ava5 cells were treated with compound **18** at indicated concentrations, and the cell lysate was subjected to western blotting with anti-HO-1 and anti-GAPDH antibodies. Data was presented as the mean ± SD of three independent experiments; $*P < 0.05$.

Figure 4. Compound 18 reduced HCV replication through inducing HO-1 expression. (A and B) The reduction effect of compound **18** on HCV replication was attenuated by SnPP treatment. Ava5 cells were co-incubated with compound **18** at 1 μM and increasing concentrations of SnPP from 0-5 μM for 3 days. Cell lysate and cellular mRNA were subjected to Western blotting and

RT-qPCR. Relative HO-1 mRNA were normalized by cellular *gapdh* mRNA levels. Data was presented as the mean ± SD of three independent experiments.

Figure 5. Compound **18** induced HO-1 expression through reduceing bach1 expression. (A) Compound **18** dose-dependently reduced bach1 expression. Ava5 cells were treated with compound 18 at indicated concentrations for 3 days. Cell lysate was subjected to western blotting with anti-Nrf2, anti-Keap-1, anti-bach1, and anti-GAPDH antibodies. (B) Compound **18** reduced the translocation of bach1 into nuclear. Ava5 cells were treated with compound **18** at indicated concentrations for 3 days. Nuclear fraction was subjected to western blotting with anti-bach1, anti-Nrf2, and anti-GAPDH antibodies. (C) Compound **18** induced the Nrf2- mediated ARE transactivation. Huh-7 and Ava5 cells were transfected with p2xARE-Luc, and the transfected-Ava5 cells were treated with compound **18** for 3 days. Cell lysate was subjected to luciferase activity assay. Data was presented as the mean ± SD of three independent experiments; $*P < 0.05$.

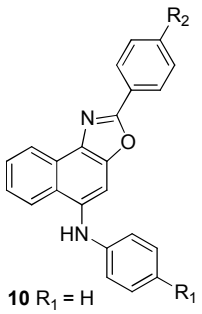
Figure 6. Compound **18** reduced HCV NS3/4A protease activity. (A) The schematic diagram of a reporter vector indicating NS3/4A protease activity. The reporter vector included the HCV NS3/4A cleavage site flanked by *egfp* and *seap* genes, named as pEG(DEΔ4AB)SEAP. (B) Compound **18** reduced NS3/4A protease activity in a concentration-dependent manner. Huh-7 cells were co-transfected with pEG(DEΔ4AB)SEAP and the NS3/4A expression vector, and the cells were treated with compound **18** for 3 days. Cell lysate was subjected to SEAP activity assay, and the fold change of NS3/4A activity was present the relative to the compound **18**-untreated control (defined as 1). Data was presented as the mean ± SD of three independent experiments; $*P < 0.05$.

Figure 7. Compound **18** induced antiviral IFN responses. (A) Compound **18** induced the activity of ISRE. Ava5 cells were transfected with pISRE-Luc, and the cells were treated with compound **18** at indicated concentrations for 3 days. The cell lysate was subjected to luciferase activity assay. (B) Compund **18** elevated IFN-α-2, IFN-α-5, and IFN-α-17 expression. (C) Compound **18** induced IFN-mediated antiviral gene expression. Ava5 cells were treated with compound **18** at indicated

concentrations for 3 days. The cellular mRNA were determined by RT-qPCR wth specific primer. Relative HCV RNA were normalized by cellular *gapdh* mRNA levels. Data was presented as the mean ± SD of three independent experiments; $*P < 0.05$.

Figure 8. Proposed action model of inhibitory effect of compound **18** on HCV replication. Compound **18** reduced bach1 expression leading to increasing the transcriptional activity of Nrf2 in the nucleus, which induced HO-1 expression. The induction of HO-1 reduced HCV NS3/4A protease activity and increased the antiviral interferon responses.

Table 1. Antiviral activities and cytotoxicities of naphtho[1,2-*d*]oxazole derivatives



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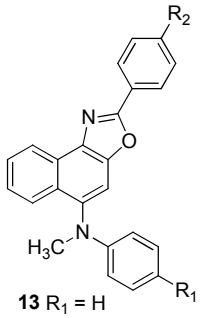
12 R_1 = OMe



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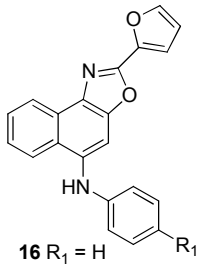

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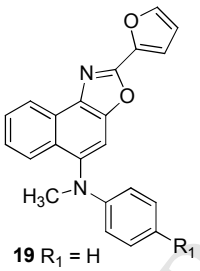


19 R_1 = H
20 R_1 = Cl
21 R_1 = OMe

compounds	R_1	R_2	HCV % Inhibition at 5 μM	HCV % Inhibition at 20 μM	Ava5 cell % viability at 20 μM	Ava5 cell % viability at 200 μM
10a	H	H	3.51 ± 1.2	4.28 ± 1.1	87.03 ± 8.03	25.52 ± 0.87
10b	H	Cl	2.50 ± 0.8	3.33 ± 3.6	99.94 ± 1.44	62.38 ± 5.19
10c	H	OMe	3.70 ± 0.5	5.31 ± 0.5	86.27 ± 1.31	68.52 ± 8.84
11a	Cl	H	1.20 ± 0.1	3.53 ± 1.1	106.90 ± 7.35	92.99 ± 1.89
11b	Cl	Cl	8.10 ± 3.2	55.20 ± 5.4	60.17 ± 3.37	27.53 ± 7.01
11c	Cl	OMe	4.50 ± 1.2	8.50 ± 1.3	83.65 ± 7.93	23.43 ± 3.67
12a	OMe	H	75.5 ± 5.5	86.67 ± 4.5	92.73 ± 6.68	37.10 ± 1.44
12b	OMe	Cl	3.20 ± 0.2	35.56 ± 1.2	102.86 ± 1.17	98.17 ± 4.88
12c	OMe	OMe	3.20 ± 0.5	5.60 ± 0.2	63.39 ± 4.49	28.49 ± 3.71
13a	H	H	1.50 ± 0.4	2.31 ± 0.4	82.29 ± 3.49	25.46 ± 1.22
13b	H	Cl	4.20 ± 1.0	5.12 ± 0.7	94.70 ± 3.78	29.58 ± 2.52
13c	H	OMe	2.50 ± 0.3	3.21 ± 0.5	69.24 ± 3.69	54.02 ± 3.18
14a	Cl	H	1.20 ± 0.3	1.21 ± 0.2	81.01 ± 2.68	25.53 ± 1.77
14b	Cl	Cl	1.10 ± 0.7	2.56 ± 3.5	86.41 ± 1.38	30.16 ± 2.57
14c	Cl	OMe	2.50 ± 0.7	6.50 ± 0.2	106.90 ± 7.35	92.99 ± 1.89
15a	OMe	H	3.40 ± 0.3	3.51 ± 0.6	83.60 ± 3.01	27.49 ± 1.20
15b	OMe	Cl	2.40 ± 0.2	2.65 ± 2.1	94.70 ± 3.78	29.58 ± 2.52
15c	OMe	OMe	5.60 ± 1.2	8.50 ± 2.6	73.61 ± 4.12	56.31 ± 2.35
16	H	--	1.80 ± 0.5	2.53 ± 2.5	81.24 ± 2.64	31.53 ± 2.87
17	Cl	--	3.20 ± 0.3	4.51 ± 0.8	83.48 ± 1.29	28.94 ± 3.12
18	OMe	--	92.10 ± 5.6	94.60 ± 1.35	89.50 ± 5.38	32.32 ± 1.78
19	H	--	2.90 ± 0.7	3.54 ± 0.5	88.83 ± 2.23	40.57 ± 5.18

20	Cl	--	2.70 ± 0.5	3.12 ± 1.3	73.01 ± 2.69	26.48 ± 1.82
21	OMe	--	2.50 ± 0.6	31.50 ± 0.3	49.50 ± 5.38	32.32 ± 1.78
ribavirin	--		31.20 ± 1.24	65.74 ± 2.19	71.37 ± 1.31	26.78 ± 1.59

Table 2. Antiviral activities [IC_{50} (μM)][a] of the compounds tested

Compounds	IC_{50}[a]	CC_{50}[b]	SI[c]
11b	17.79 ± 1.78	83.74 ± 4.58	4.71
12a	3.27 ± 0.93	158.24 ± 5.96	48.39
18	0.63 ± 0.07	144.33 ± 3.89	229.10
ribavirin	13.16 ± 1.63	106.27 ± 3.69	8.08

[a] The IC_{50} is the concentration of the compound resulting in a 50% inhibition in virus production.

[b] The CC_{50} is the concentration of the compound causing a 50% growth inhibition of uninfected ava-5 cells.

[c]SI: selectivity index. SI = CC_{50}/IC_{50}.

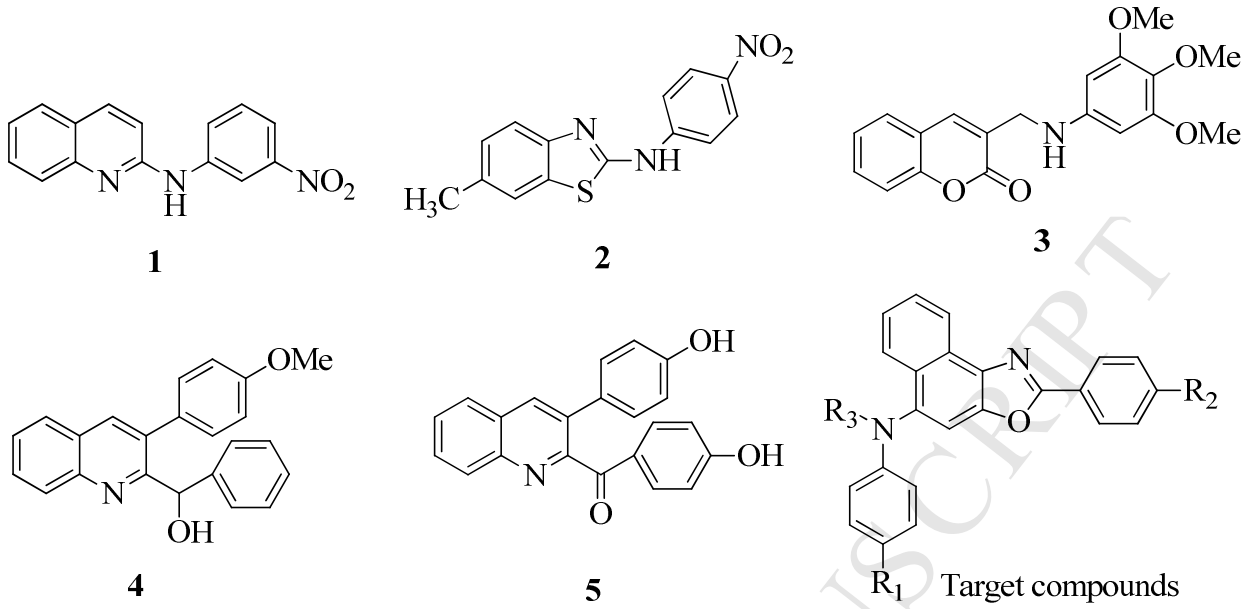


Figure 1.

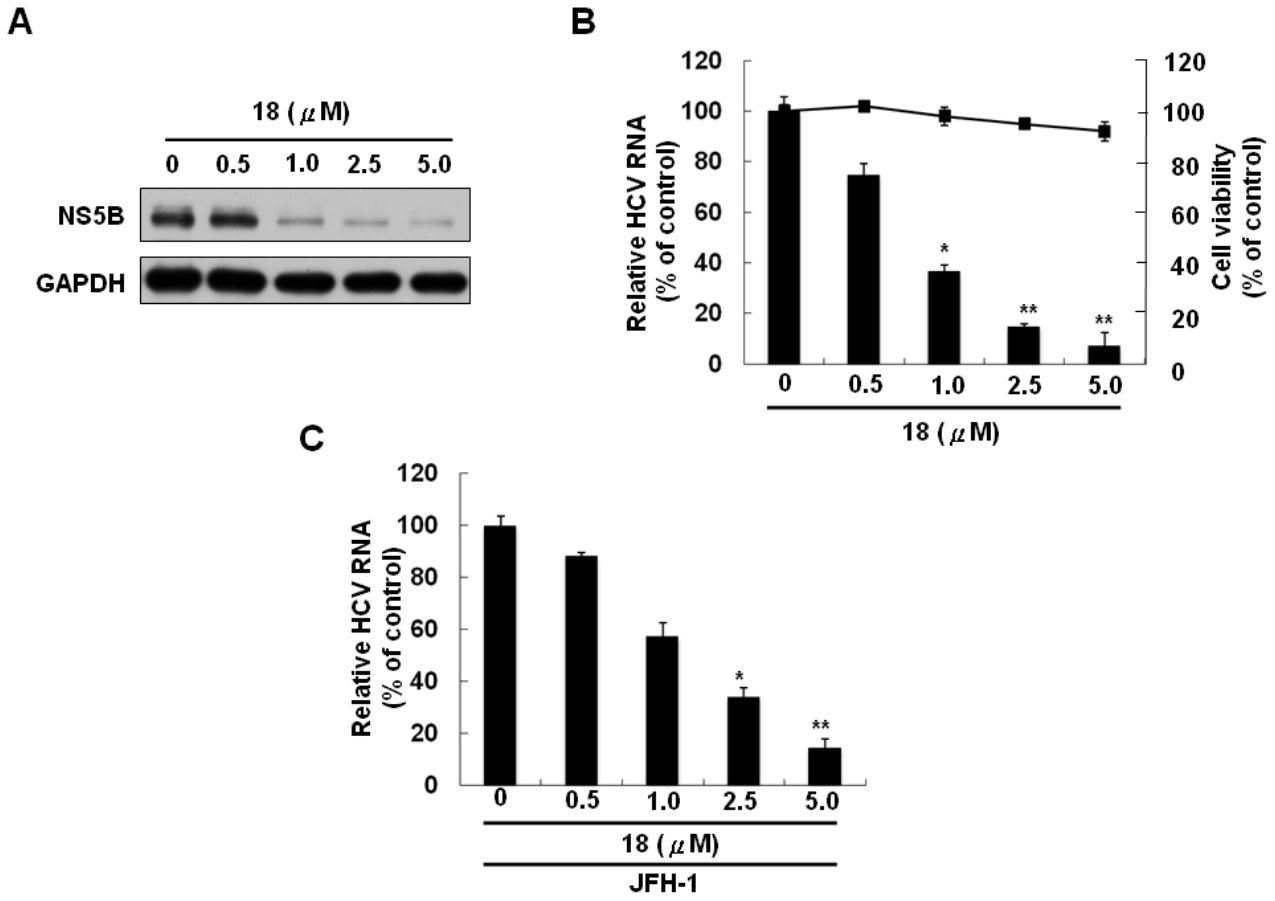


Figure 2.

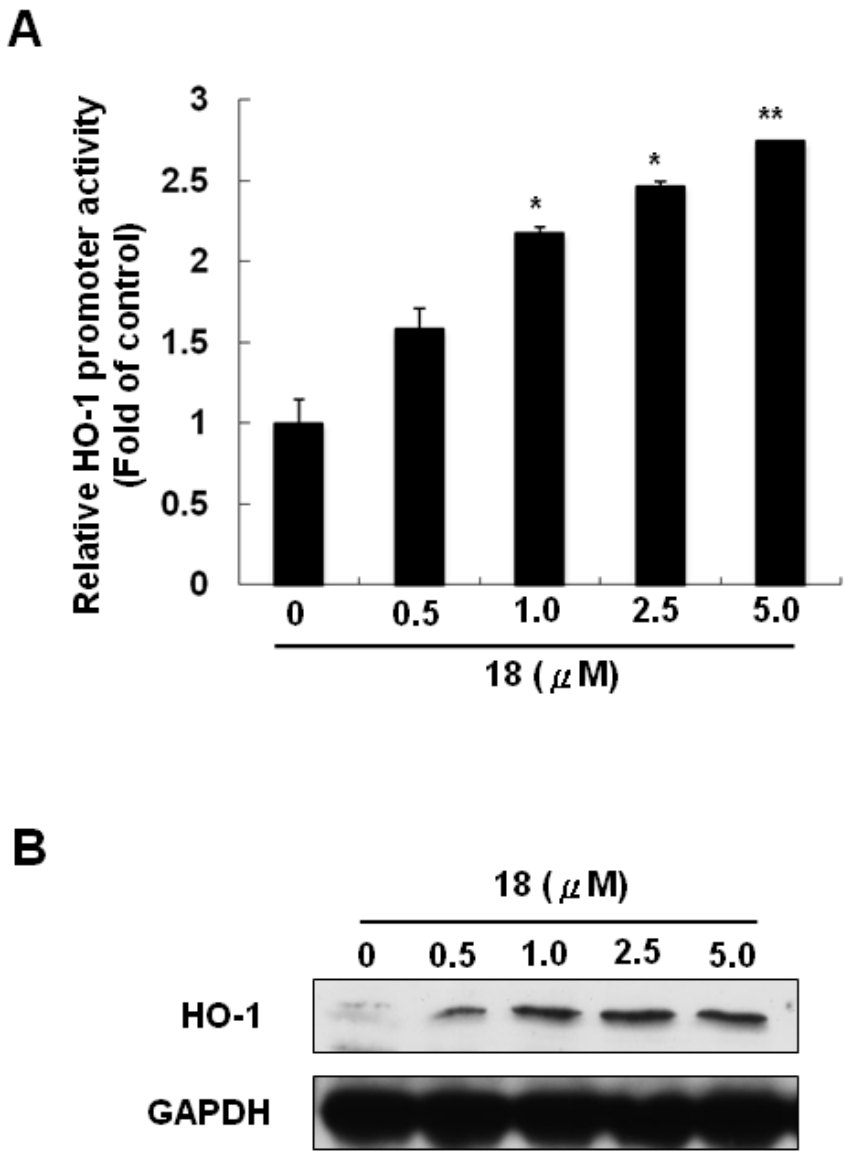
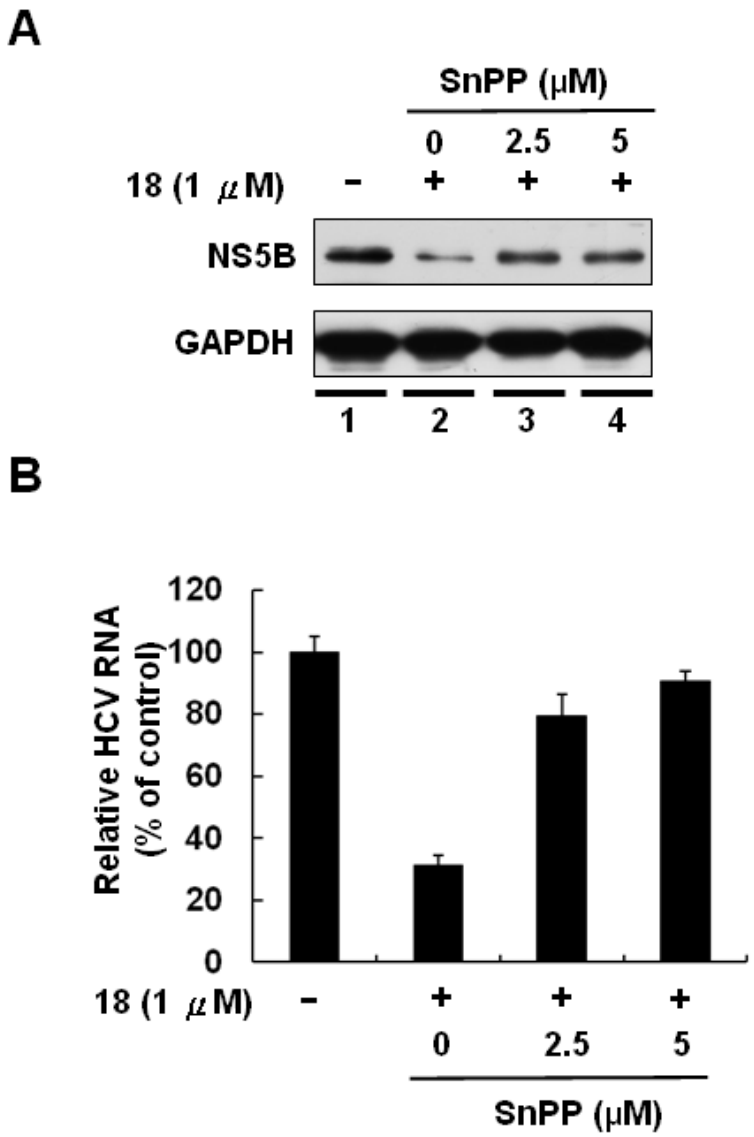


Figure 3.



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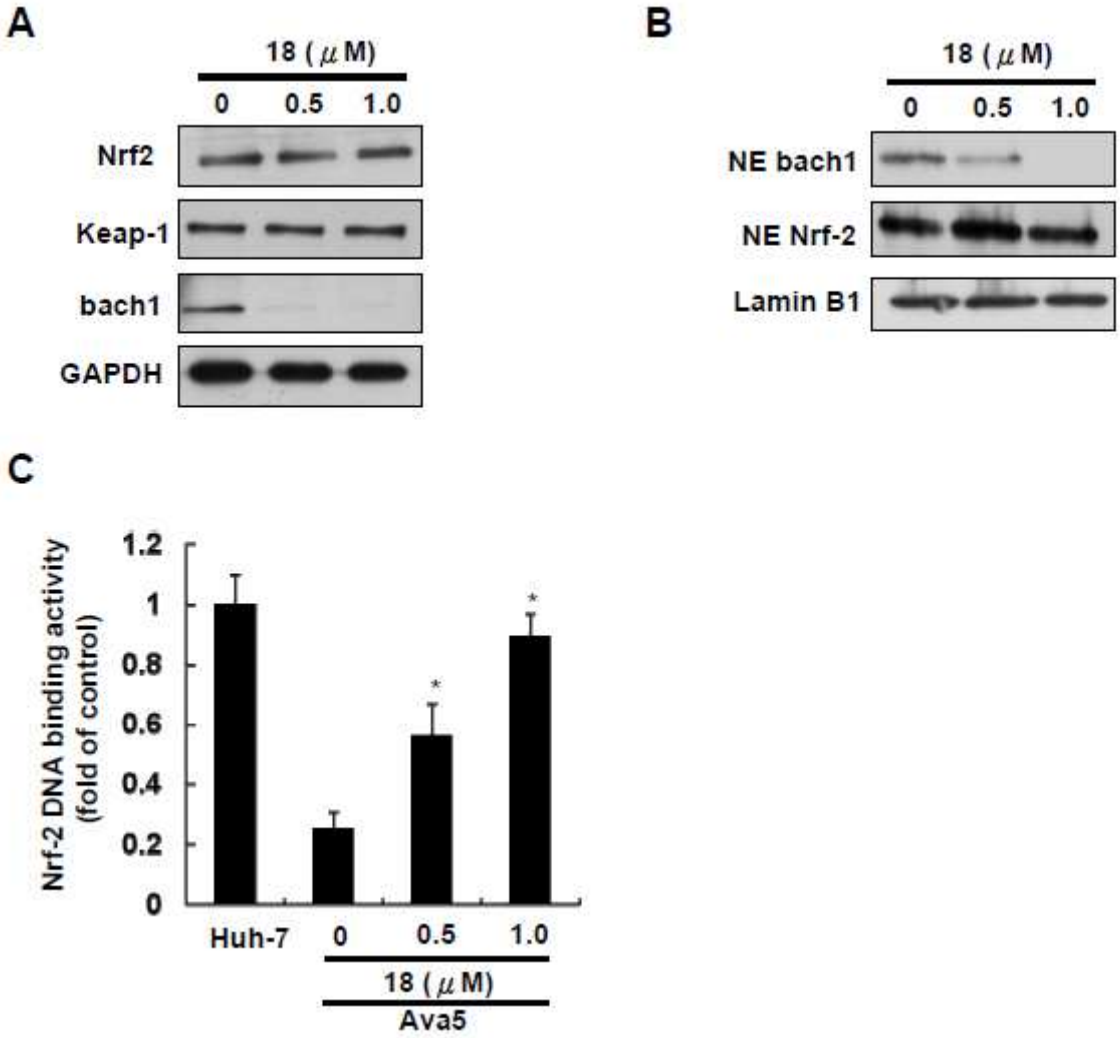
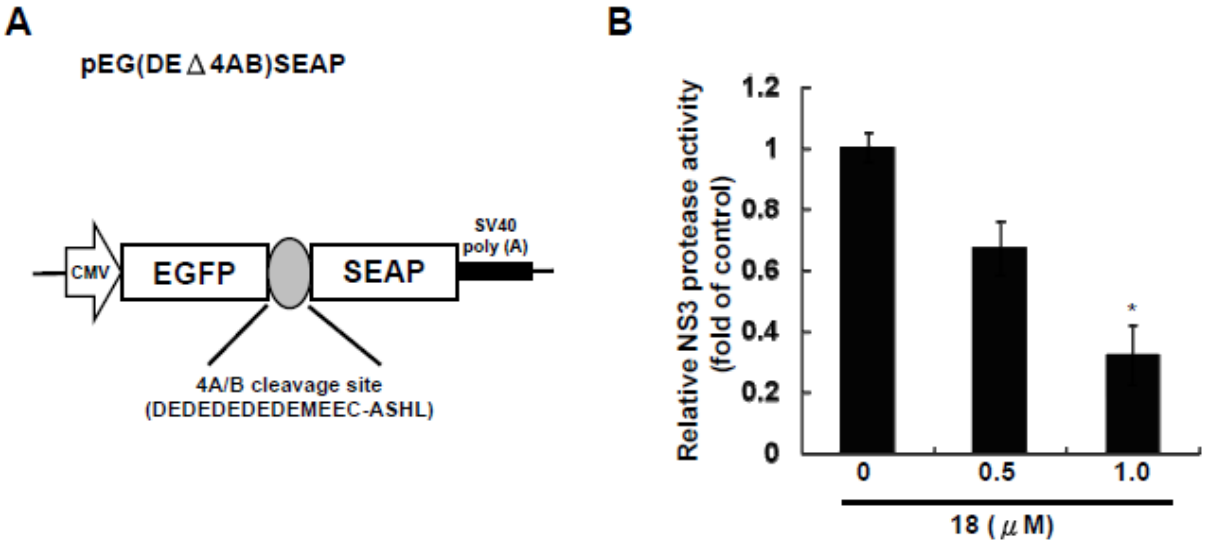


Figure 5.



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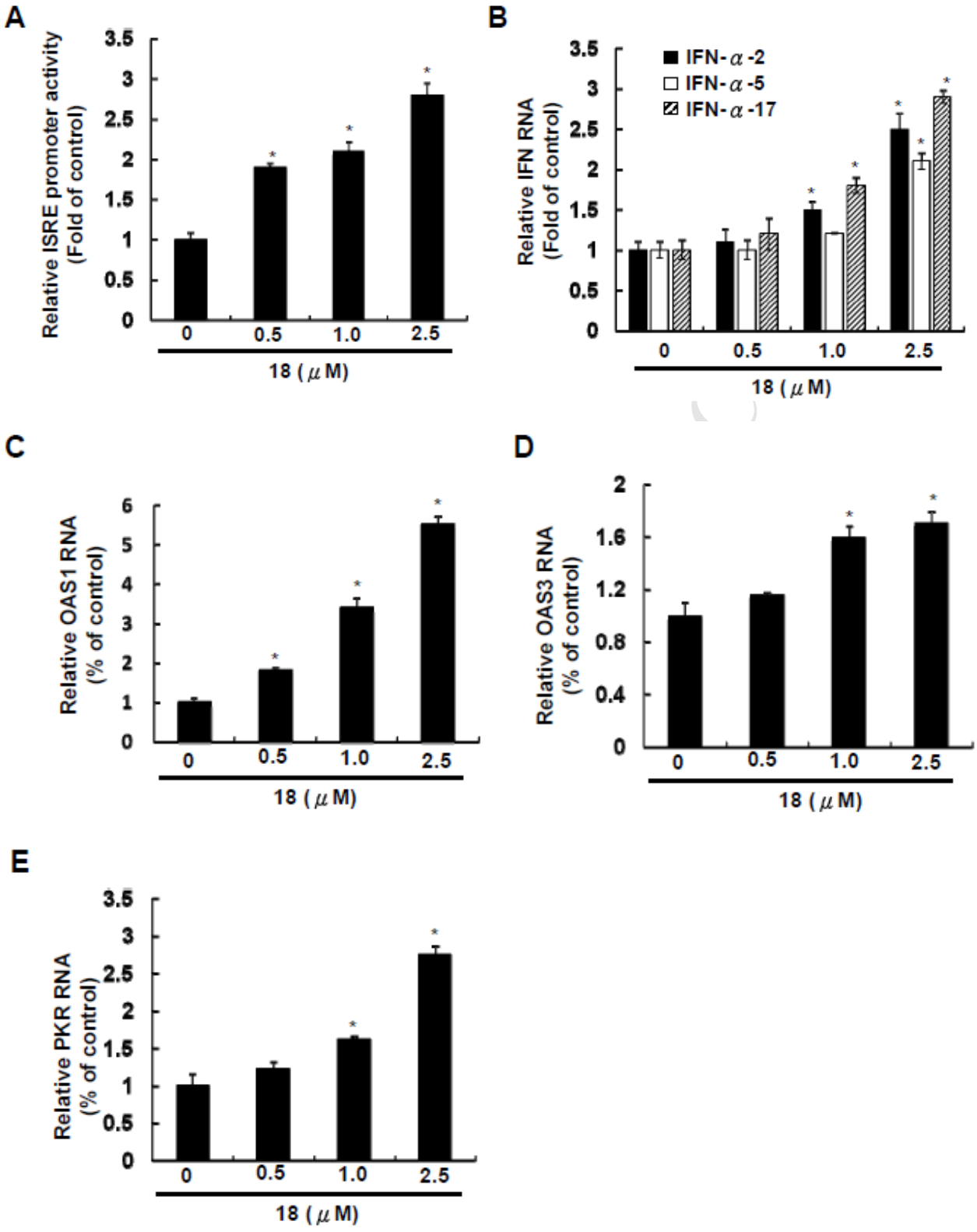

A
Relative ISRE promoter activity (Fold of control)
3.5
3
2.5
2
1.5
1
0.5
0
0
0.5
1.0
2.5
18 (μM)
B
Relative IFN RNA (Fold of control)
IFN-α-2
IFN-α-5
IFN-α-17
3.5
3
2.5
2
1.5
1
0.5
0
0
0.5
1.0
2.5
18 (μM)
C
Relative OAS1 RNA (% of control)
6
5
4
3
2
1
0
0
0.5
1.0
2.5
18 (μM)
D
Relative OAS3 RNA (% of control)
2
1.6
1.2
0.8
0.4
0
0
0.5
1.0
2.5
18 (μM)
E
Relative PKR RNA (% of control)
3.5
3
2.5
2
1.5
1
0.5
0
0
0.5
1.0
2.5
18 (μM)

Figure 7.

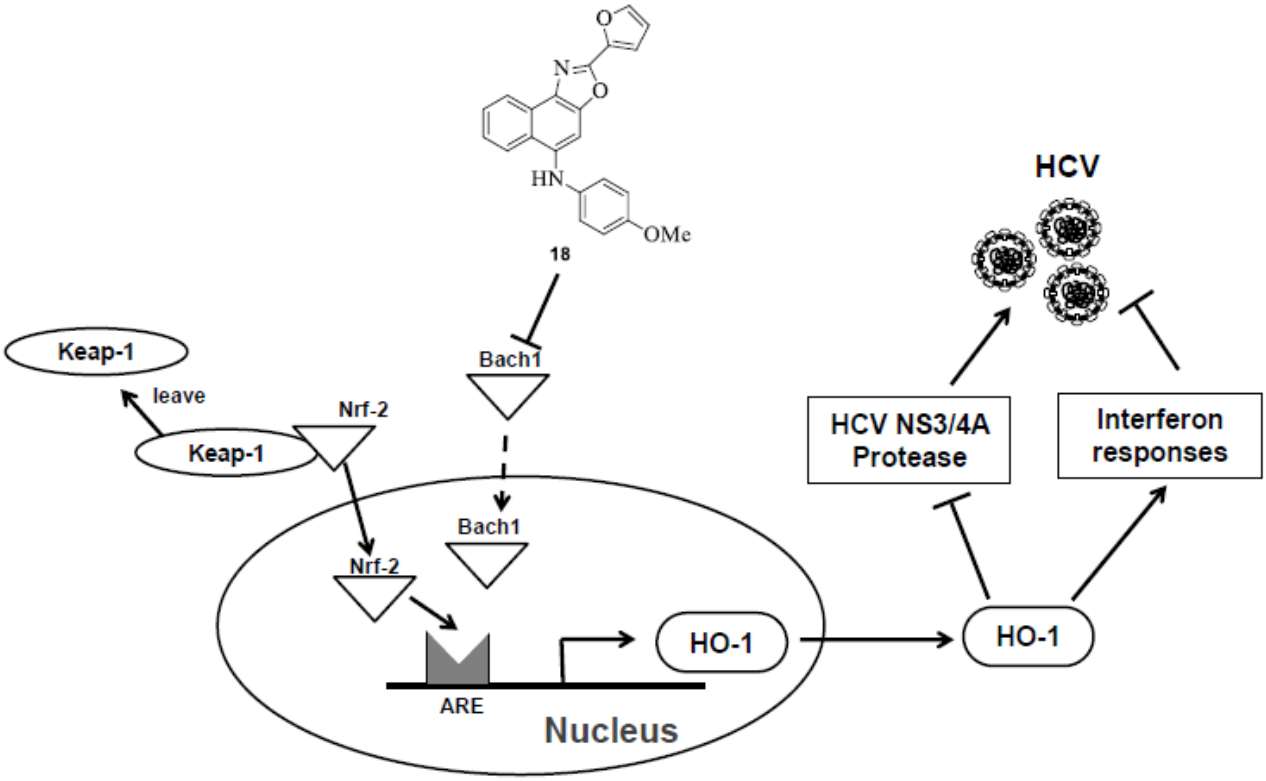
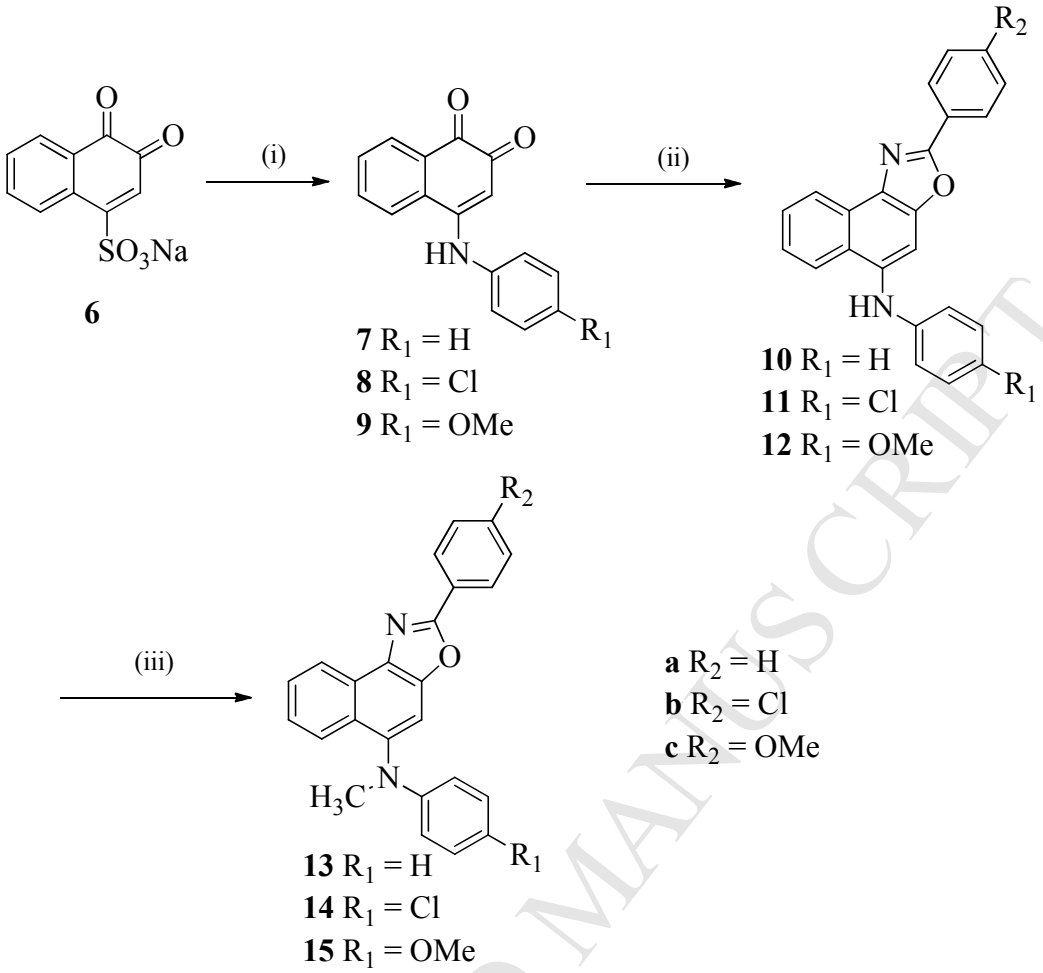
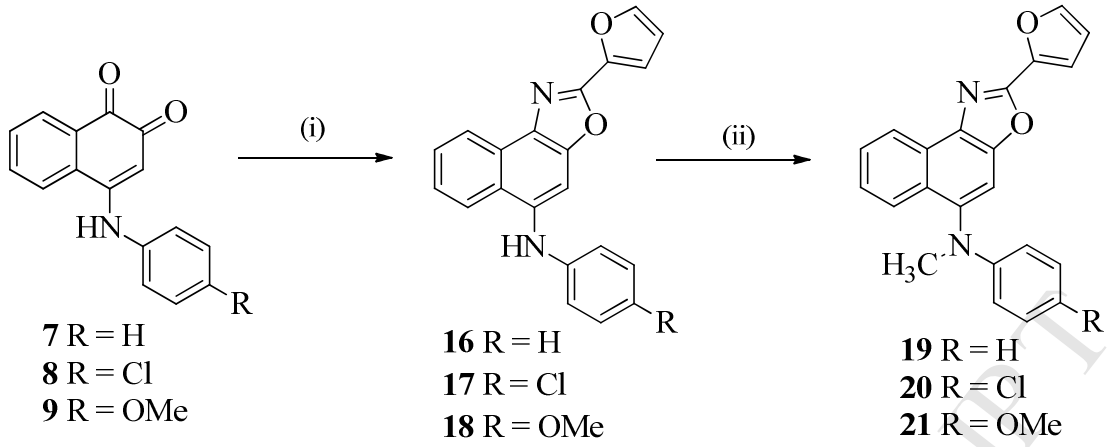


Figure 8.



Scheme 1: Reagents and conditions: (i) substituted aniline, H_2O, rt, 20 min; (ii) substituted aldehyde, NH_4OAc, AcOH, 150^oC, 2.5h (iii)MeI, NaH, dry DMF, 10 min.



7 R = H
8 R = Cl
9 R = OMe

16 R = H
17 R = Cl
18 R = OMe

19 R = H
20 R = Cl
21 R = OMe

Scheme 2: Reagents and conditions: (i) furfural, NH_4OAc, AcOH, 150°C, 2.5h (ii) MeI, NaH, dry DMF, 10 min.

Highlights

▲ Naphtho[1,2-*d*]oxazole compounds were synthesized.

▲ **18** was more anti-HCV activity (IC_{50} = 0.63 μM) than ribavirin (IC_{50} = 13.16 μM)

▲ The selective index (SI) of **18** is approximately 28-folds higher than ribavirin.

▲ **18** reduced bach1 expression resulting in increasing the activity of Nrf-2 binding element.

▲ The induction of HO-1 by compound **18** reduced HCV NS3/4A protease activity and induced the antiviral interferon response.