

Synthesis and Bioactivity of *N*-(4-Chlorophenyl)-4-Methoxy-3-(Methylamino) Benzamide as a Potential Anti-HBV Agent

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Introduction: Hepatitis B virus (HBV) is a global health concern that can cause acute and chronic liver diseases. Thus, there is an urgent need to research novel anti-HBV agents. Our previous reports show that *N*-phenylbenzamide derivatives exert broad-spectrum antiviral effects against HIV-1, HCV, and EV71 by increasing intracellular levels of APOBEC3G (A3G). As A3G is capable of inhibiting the replication of HBV, we screened the *N*-phenylbenzamide derivatives against HBV.

Methods: In this study, a new derivative, *N*-(4-chlorophenyl)-4-methoxy-3-(methylamino) benzamide (IMB-0523), was synthesized and its anti-HBV activity was evaluated in vitro and in vivo. The acute toxicity and pharmacokinetic profiles of IMB-0523 were also investigated.

Results: Our results show that IMB-0523 has higher anti-HBV activity in both wild-type HBV (IC₅₀: 1.99 μM) and drug-resistant HBV (IC₅₀: 3.30 μM) than lamivudine (3TC, IC₅₀: 7.37 μM in wild-type HBV, IC₅₀: >440 μM in drug-resistant HBV). The antiviral effect of IMB-0523 against HBV may be due to an increased level of intracellular A3G. IMB-0523 also showed low acute toxicity (LD₅₀: 448 mg/kg) in mice and promising PK properties (AUC_{0-t}: 7535.10±2226.73 μg·h/L) in rats. Further, IMB-0523 showed potent anti-HBV activity in DHBV-infected ducks.

Conclusion: Thus, IMB-0523 may be a potential anti-HBV agent with different mechanisms than current anti-HBV treatment options.

Keywords: anti-HBV activity, APOBEC3G, hepatitis B virus, IMB-0523, PK, toxicity

Introduction

Hepatitis B virus (HBV) infection can cause acute or chronic liver diseases and has long been a serious public health problem. The World Health Organization (WHO) reports that approximately 257 million people are chronically infected with HBV, and approximately 887,000 people die from HBV-related complications annually.^{1,2} This virus belongs to a species in the Hepadnaviridae family, which also includes other viruses such as woodchuck HBV (WHV) and duck HBV (DHBV). Multiple drugs are used to treat HBV, including interferons (IFNs) and nucleos(t)ide analogues such as lamivudine (3TC), tenofovir (TFV), and entecavir (ETV).^{3,4} Unfortunately, while these drugs rarely cure the viral infection, they are known to cause side effects and drug resistance.^{5,6} Thus, there is an urgent need to research novel agents with different anti-HBV mechanisms.^{7,8}

The apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3 (APOBEC3; A3) family members (A3A-A3H) encode DNA cytidine deaminases,

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which have antiviral effects against DNA and RNA viruses. APOBEC3G (A3G) is a potent host defence factor, which inhibits HIV-1 replication via its G-to-A hypermutation activity.⁹ HBV genome editing by A3G has also been reported.^{10,11} In addition, A3G can be packaged into HBV nucleocapsids and inhibit HBV DNA reverse transcription in a deaminase-independent manner.^{12–14} The ability of A3G to block viral replication extends even to drug-resistant strains of HIV.^{9–18} Thus, A3G may be a useful target for antiviral therapy and provide a new approach to developing anti-HBV drugs, especially for drug-resistant strains of HBV.

In the previous studies, we described a series of *N*-phenylbenzamide derivatives (Figure 1) that have broad-spectrum antiviral effects against HIV-1 (IMB-26 and IMB-35), HCV (IMB-26), and EV71 (IMB-Z). The primary mechanism of antiviral effects is through increasing intracellular levels of A3G.^{15–17} As A3G is capable of inhibiting the replication of HBV, we screened the *N*-phenylbenzamide derivatives against HBV. Due to alkylation of the amine group of benzene ring A, the synthesized derivatives are metabolically stable. In this study, we found that a novel derivative, *N*-(4-chlorophenyl)-4-methoxy-3-(methylamino) benzamide (IMB-0523) is an active inhibitor of both wild-type and drug-resistant HBV; intracellular A3G level was also determined in HepG2.2.15 cells. Additionally, acute toxicity and pharmacokinetic (PK) profiles of IMB-0523 were investigated in mice and rats, respectively. Further, because the duck HBV (DHBV) model is widely used to study HBV in vivo,^{19–21} we also examined the in vivo anti-HBV activity of IMB-0523 using the DHBV animal model.

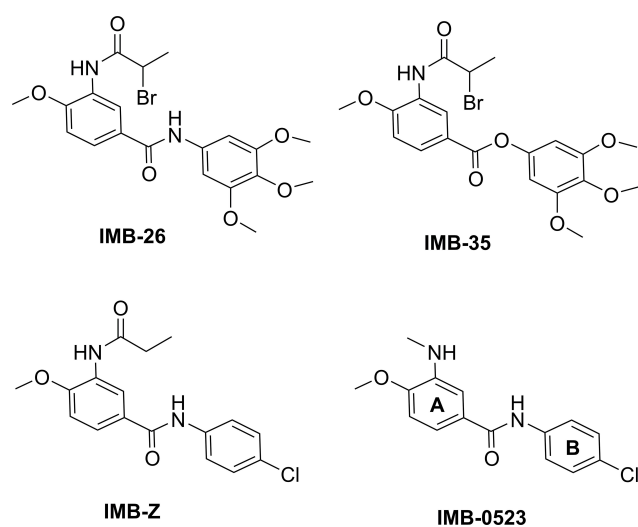


Figure 1 The structures of *N*-phenylbenzamide derivatives IMB-26, IMB-35, IMB-Z, and IMB-0523.

Thus we identified a *N*-phenylbenzamide derivative compound IMB-0523 that could inhibit HBV replication and its antiviral activity may be associated with the increase of intracellular A3G. Although the detailed mechanism remains to be further investigated, our results thus warrant further development of *N*-phenylbenzamide derivatives as antiviral agents to treat diseases caused by HBV infection and provided a potential therapeutic approach for drug resistance due to its different mechanisms with current anti-HBV drugs.

Materials and Methods

Materials

3-Amino-4-methoxybenzoic acid, dimethyl sulphate, 4-chloroaniline, *N,N*-diisopropylcarbodiimide (DIC), and *N*-hydroxybenzotriazole (HOBt) were obtained from J&K Scientific (Beijing, China). 1,1,2,2-Tetrachloroethane (Cl₂CH₂CH₂Cl₂), sodium hydroxide (NaOH), hydrochloric acid (HCl), and acetic acid (HOAc) were obtained from Beijing Chemical Works (Beijing, China).

Chemistry

¹H NMR and ¹³C NMR spectra were recorded on a Bruker 400 MHz spectrometer (Bruker, Ettlingen, Germany). Melting point was determined with an MP90 melting point system (Mettler-Toledo AG, Switzerland). High-resolution mass spectra were recorded using an LTQ Orbitrap XL instrument (Thermo Fisher Scientific, San Jose, CA, USA). Analytical high-performance liquid chromatography (HPLC) was performed on an Agilent Technologies 1200 Series Instrument. The compound was analyzed using an Eclipse XDB-C18 (4.6×250 mm, 5 μm) under a 254 nm UV detector. Methanol - water (85:15) was used as mobile phase at a flow rate of 1 mL/min.

Synthesis of IMB-0523

3-Amino-4-methoxybenzoic acid (50.1 g, 0.3 mol) was dissolved in 30% NaOH/H₂O (150 mL), and 42 mL dimethyl sulphate (0.45 mol) was slowly added. The resulting mixture was stirred for 24 h at room temperature. The pH was adjusted to 5 by adding diluted acetic acid and the mixture was stirred for 30 min to precipitate solids. The mixture was filtered, and the wet filter cake was recrystallized from 600 mL methanol to give the compound 1a in 43% yield (23.2 g). Next, 1a (6.72 g, 0.037 mol) was dissolved in Cl₂CH₂CH₂Cl₂ (50 mL), and DIC (6.94 g, 0.056 mol) and HOBt (7.50 g, 0.056 mol) were added to the solution. The resulting mixture was stirred for 1 h at room temperature, after which 4-chloroaniline (6.20 g, 0.048

mol) was added. The mixture was stirred for 10 h at room temperature and concentrated in vacuo; 100 mL ethyl acetate was then added. The organic layer was successively washed with 0.5 N NaOH solution (30 mL), 0.5 N HCl solution (30 mL), and brine (30 mL). The solution was then dried over anhydrous MgSO₄, filtered and concentrated, and the crude residue was recrystallized from 100 mL ethyl acetate to give the IMB-0523 in 32% yield (3.5 g).

N-(4-chlorophenyl)-4-methoxy-3-(methylamino) benzamide (IMB-0523). White solid, yield: 13.8%. mp: 170–171 °C. Purity 98.5%. ¹H NMR (400 MHz, DMSO-d₆) δ 10.10 (s, 1H), 7.85–7.79 (m, 2H), 7.42–7.36 (m, 2H), 7.27 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.04 (d, *J* = 1.9 Hz, 1H), 6.91 (d, *J* = 8.4 Hz, 1H), 5.24 (s, 1H), 3.86 (s, 3H), 2.80 (s, 3H). ¹³C NMR (101 MHz, DMSO-d₆) δ 166.42, 149.66, 139.50, 138.98, 128.86, 127.76, 127.28, 122.28, 116.21, 108.98, 107.89, 56.01, 30.20. High resolution mass spectra (HRMS) *m/z*: 291.0894 [M+H]⁺, Calcd for C₁₅H₁₆N₂O₂ Cl: 291.0894. ([Supplementary data page 1, 2](#))

Anti-HBV Effect in vitro

HBV-replicating cell line HepG2.2.15 (wild-type) was obtained from Mount Sinai Medical Center, USA. HepG2. A64 (ETV and 3TC-resistant HBV) cell line was a gift of Dongping Xu from No.302 Hospital of the People's Liberation Army, China. The use of the cell lines have been approved by our institute (Institute of Medicinal Biotechnology) under biosafety level 2 (BSL-2) conditions. HepG2.2.15 cells and HepG2. A64 cells were seeded at 1×10⁵ cells/mL in 96-well plates and incubated at 37 °C for 24 h. Cells were treated with a series concentration of IMB-0523 every 3 days for 6 days in a time-dependent experiment; 3TC was used as the positive control. After 6 days, the percentage of cell death after drug treatment was measured using an MTT assay. Cytotoxicity of these compounds was expressed as the concentration of IMB-0523 required to inhibit 50% (CC₅₀) each of the HepG2.2.15 cells and HepG2. A64 cells. Intracellular HBV DNA was extracted and quantified with a quantitative PCR assay. The antiviral efficacy of compound was expressed as the concentration that inhibited the amount of HBV DNA by 50% (IC₅₀) in comparison with the levels of the mock-treated controls.

Western Blot Assay

The cellular proteins were extracted using M-PER Mammalian Protein Extraction Reagent containing Halt Protease Inhibitor Single-Use cocktail; the extracted proteins were denatured by adding 5× sodium dodecyl sulphate–

polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer (all purchased from Thermo Scientific, Rockford, IL, USA), followed by boiling for 5 min at 100 °C. Approximately 10 µg of the extracted proteins were applied to SDS-PAGE. The primary antibody against β-actin (Cell Signaling Technology, Beverly, MA, USA) and A3G (Abcam, Cambridge, MA, USA) were used.

Acute Toxicity Assay

ICR mice (weight = 18–22 g; age = 4 weeks; n = 6 mice per group; 3 males, 3 females) were purchased from Vital River Laboratories (Beijing, China). Mice were injected with 500, 385, 296, and 228 mg/kg IMB-0523 intraperitoneally in a single-dose experiment. Survival and body weight were monitored daily for 7 days. The median lethal dose (LD₅₀) of IMB-0523 was calculated according to the Karber method. All animal experiments were performed in full compliance with the protocols approved by the Animal Ethics Committee of the Institute of Medicinal Biotechnology (Beijing, China). All procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committees of the Chinese Academy of Medical Sciences.

Pharmacokinetics Study

Three male SD rats (weight = 180–220 g; age = 4 weeks) were purchased from Vital River Laboratories (Beijing, China). Each rat was dosed with a single oral administration of 20 mg/kg IMB-0523. Blood samples (about 0.5 mL) were collected at 0.08, 0.25, 0.50, 1, 2, 3, 4, 6, 8, 12, and 24 h, and were centrifuged for 10 min to obtain plasma sample. The separated plasma samples were stored at –20 °C for analysis. Next, 50 µL of plasma sample was added to 150 µL of methanol and centrifuged twice at 14,000 g for 5 min. The LC-MS method was used to quantify the IMB-0523 in rat plasma samples. Five microliters of serum sample was injected on an Agilent ZORBAX Extend-C18 column (2.1×50 mm, 3.5 µm). Mobile phase A - mobile phase B (30:70) was used as mobile phase at a flow rate of 0.2 mL/min. Mobile phase A was 0.1% formic acid in water and mobile phase B was 0.1% formic acid in methanol. The positive protonated molecular ions of IMB-0523 was monitored at 291 [M+H]⁺. The PK parameters were determined by a non-compartmental analysis method using Phoenix WinNonlin software (Pharsight Corporation, CA, USA).

Antiviral Efficacy in a Duck HBV Model

All procedures with animal experiments were conducted according to the standard operating procedures approved by

the Institutional Animal Ethics Committee under animal BSL-2 (ABSL-2) conditions. All procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committees of the Chinese Academy of Medical Sciences. Pekin ducklings were obtained from Beijing Qianjin Duck Farm (Beijing, China) and experimentally infected with DHBV-positive duck serum. Ducks were tested for DNA load 7 days post-injection (T0) and divided into four groups ($n = 5$ per group), which were treated twice daily with 25, 50 or 100 mg/kg of IMB-0523 via oral gavage. One group of ducks was treated with 50 mg/kg of 3TC once daily as positive control. Ducks were treated daily for 14 days, and monitored for three additional days to assess the rebound of viral replication after treatment cessation. Blood was collected on days 0 (T0), 7 (T7), and 14 (T14) of treatment, and 3 days (P3) after treatment cessation. Serum DHBV DNA was extracted and quantified by a quantitative PCR assay using TransStart Tip Green qPCR SuperMix (TransGen Biotech, Beijing, China).

Results and Discussion

Chemistry of IMB-0523 Synthesis

The synthesis of IMB-0523 is shown in [Scheme 1](#). Synthesis of IMB-0523 comprises two steps: firstly, 3-amino-4-methoxybenzoic acid was alkylated by dimethyl sulphate to afford the intermediate compound **1a**; then, compound **1a** was condensed with 4-chloroaniline using *N,N*-diisopropylcarbodiimide (DIC) as a coupling reagent and *N*-hydroxybenzotriazole (HOBT) as an activating reagent, to yield IMB-0523. The structure is confirmed by HPLC, HRMS, and NMR ([Supplementary data page 1, 2](#)).

Anti-HBV Activity in vitro

IMB-0523 was screened for inhibitory in vitro effects on the replication of HBV in HepG2.2.15 (wild-type) and HepG2. A64 (3TC and ETV-resistant) cells by detecting the levels of intracellular HBV DNA. The IC_{50} , CC_{50} , and SI values of IMB-0523 are shown in [Table 1](#). IMB-0523

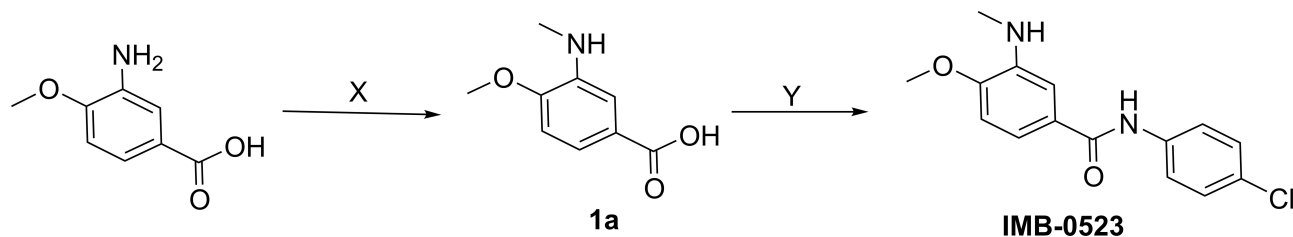
showed a potent anti-HBV activity in both HepG2.2.15 (IC_{50} : 1.99 μ M) and HepG2. A64 (IC_{50} : 3.30 μ M) cells while lamivudine (3TC, IC_{50} : 7.37 μ M in wild-type HBV, IC_{50} : >440 μ M in drug-resistant HBV) only exhibited a significant inhibitory effect on HBV DNA levels in HepG2.2.15 cells. The SI values were similar in HepG2.2.15 (SI: 58) and HepG2. A64 (SI: 52). These results indicate that IMB-0523 has potential anti-HBV activity against both wild-type HBV (IC_{50} : 1.99 μ M, SI: 58) and drug-resistant HBV (IC_{50} : 3.30 μ M, SI: 52).

The Level of Intracellular A3G

As A3G is a potentially useful target for antiviral therapy and in developing broad-spectrum antivirals,^{9–18} we determined the intracellular A3G level in HepG2.2.15 cells. The results show that IMB-0523 treatment increased the level of A3G protein in HepG2.2.15 cells in a concentration-dependent manner ([Figure 2](#)). It was previously reported that some *N*-phenylbenzamide derivatives inhibit the replication of HIV-1, HCV, and EV71 by increasing intracellular levels of A3G.^{15–17} Antiviral proteins among APOBEC3 family can restrict viral replication via cytidine deaminase-dependent and independent mechanisms and A3G inhibits HBV DNA replication in a deaminase independent way.¹² It has been reported that A3G directly binds HBV core protein and can be packaged into HBV nucleocapsids.^{13,22} As A3G is a potent host restriction factor that blocks HBV replication, we speculated that the antiviral effect of IMB-0523 against HBV may be due to increased levels of intracellular A3G. The detailed mechanisms of how IMB-0523 restricts HBV DNA replication through inducing A3G expression will be further studied.

Acute Toxicity Study in Mice

The four groups of ICR mice were evaluated after intraperitoneal injection of varying doses of IMB-0523. Survival and body weight were monitored daily for 7 days. No statistically significant weight change was



Scheme 1 Synthesis of IMB-0523. Reagents and conditions: (X) dimethyl sulphate, NaOH, H₂O; r. t., 24 h; HOAc, H₂O. (Y) 4-chloroaniline, DIC, HOBT, Cl₂CH₂CH₂Cl₂; r. t., 10 h.

Table 1 In vitro Anti-HBV Activity of IMB-0523

Compd.	Cell Lines	IC ₅₀ ^a (μM)	CC ₅₀ ^b (μM)	SI ^c
IMB-0523	HepG2.2.15	1.99	116.01	58
	HepG2. A64	3.30	171.93	52
3TC	HepG2.2.15	7.37	>1000	>136
	HepG2. A64	>440	>1000	-

Notes: ^aIC₅₀: 50% inhibitory concentration of intracellular HBV DNA synthesis. ^bCC₅₀: 50% cytotoxic concentration on HepG2.2.15 cells and HepG2. A64 cells. ^cSI: selective index (CC₅₀/IC₅₀).

observed during treatment (data not shown). The median lethal dose (LD₅₀ value) was determined by the Karber method (Table 2). The LD₅₀ of IMB-0523 in mice was 448 mg/kg via intraperitoneal injection. These results indicate that IMB-0523 has low acute toxicity in mice.

Pharmacokinetic Profiles of IMB-0523

We evaluated the in vivo PK profile of IMB-0523 in male Sprague Dawley (SD) rats by administering a single oral dose of 20 mg/kg IMB-0523. The presence of IMB-0523 in rat plasma samples was determined by liquid chromatography with tandem mass spectrometry (LC-MS), and the PK parameters were determined by a non-compartmental analysis method using Phoenix WinNonlin software (Pharsight Corporation, CA, USA). The PK results are presented in Tables 3 and S1 (Supplementary data page 3). IMB-0523 showed acceptable PK parameters, with AUC_{0-t} 7535.10 ± 2226.73 μg·h/L, MRT_{0-t} 1.90 ± 0.31 h, t_{max} 0.75 ± 0.43 h, and C_{max} 2690.17 ± 574.31 μg/L. These results indicate that IMB-0523 is sufficiently metabolically stable to support study of its in vivo activity in an animal model. Metabolic stability of IMB-0523 may due to alkylation of the amine group of benzene ring A.

Anti-HBV Activity in vivo

To determine the in vivo anti-HBV activity of IMB-0523, DHBV-infected ducks were treated with 25, 50 and 100 mg/

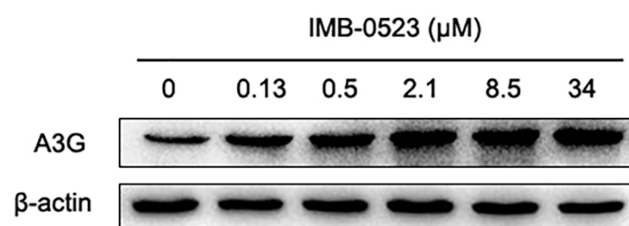


Figure 2 IMB-0523 treatment increases the level of intracellular A3G. Cells were treated with indicated concentrations of IMB-0523 and harvested after 6 days post-treatment. The amounts of cellular A3G and β-actin proteins (μM) were determined by Western blot assay.

Table 2 Acute Toxicity (Median Lethal Dose, LD₅₀) of IMB-0523

Compd.	Dose (mg/kg)	Death Rate (%)	LD ₅₀ (mg/kg) (95% Confidence Interval)
IMB-0523	500	66.7%	448 (384–549)
	385	16.7%	
	296	33.3%	
	228	0%	

kg of IMB-0523 orally; 50 mg/kg of 3TC was used as the positive control and saline was used as the negative control. Duck blood samples were collected at days 0 (before treatment, T0), 7 (T7), and 14 (T14) of treatment, and at day 3 after the termination of treatment (P3) to quantify DHBV DNA by a quantitative PCR assay (Figure 3). For 50 mg/kg of 3TC, the serum DHBV DNA in ducks was significantly inhibited at T7 ($P < 0.001$) and T14 ($P < 0.001$). For both 25 mg/kg and 50 mg/kg of IMB-0523, the serum DHBV DNA in ducks was significantly inhibited at T14 ($P < 0.001$). For 100 mg/kg of IMB-0523, the serum DHBV DNA in ducks was significantly inhibited at T7 ($P < 0.001$) and T14 ($P < 0.001$). Noticeably, 100 mg/kg of IMB-0523 treatment induced a similar reduction of serum DHBV DNA to 3TC treatment up to 2 log₁₀ compared to vehicle controls at T7. As a nucleos(t)ide analogue which can be incorporated into the growing DNA chain by competing with the natural nucleotide substrates, 3TC usually induces a great reduction in serum DHBV DNA in vivo. However, the antiviral activities of IMB-0523, which targets the host factor, in vitro and in vivo may be different to some extent. Overall, our results indicate that IMB-0523 shows potent antiviral activity in a duck HBV model.

Conclusion

In summary, IMB-0523 was synthesized and the anti-HBV activities were evaluated in vitro and in vivo. Acute toxicity and pharmacokinetic profiles of IMB-0523 were also

Table 3 Pharmacokinetic Properties of IMB-0523 in SD Rats (n=3)

Parameter	IMB-0523
AUC _{0-t} (μg·h/L) ^a	7535.10 ± 2226.73
MRT _{0-t} (h) ^b	1.90 ± 0.31
T _{max} (h) ^c	0.75 ± 0.43
C _{max} (μg/L) ^d	2690.17 ± 574.31

Notes: ^aTotal area under the concentration-time curve. ^bMean residence time. ^cThe time at which C_{max} observed. ^dMaximum serum concentration.

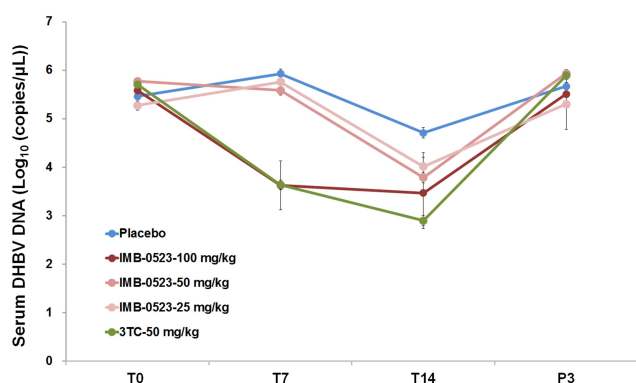


Figure 3 In vivo antiviral activity of IMB-0523 in a duck HBV model. DHBV-infected ducks were treated with three doses of IMB-0523 at 25 mg/kg, 50 mg/kg, and 100 mg/kg or the vehicle via oral gavage daily. 3TC treatment serves as positive controls. Five duck were included in each group. Blood was collected on day 0 (T0, before treatment), 7 (T7) and 14 (T14) days posttreatment and 3 days after treatment cessation (P3) and serum DHBV DNA was extracted and analyzed by a quantitative PCR assay. Mean values \pm SD are plotted for each group (n=5).

investigated. IMB-0523 showed potential anti-HBV activity and low toxicity in both wild-type and drug-resistant HBV. The antiviral effect of IMB-0523 HBV may be due to an increase in intracellular A3G levels. IMB-0523 showed low acute toxicity in mice and promising PK properties in rats. Additionally, IMB-0523 showed potent anti-HBV activity during treatment. Thus, IMB-0523 may be a potential anti-HBV agent with anti-HBV mechanisms that differ from current treatments and is a promising candidate for further anti-HBV drug discovery.

Acknowledgments

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Disclosure

Dr Si-Tu Xue and Professor Zhuo-Rong Li report a patent China National Intellectual Property Administration licensed to Institute of Medicinal Biotechnology Chinese Academy of Medical Sciences. The authors report no other potential conflicts of interest in this work.

References

- Burwitz BJ, Hashiguchi PK, Mansouri M, et al. MHC-E-restricted CD8 +T cells target Hepatitis B virus-infected human hepatocytes. *J Immunol.* 2020;204(8):2169–2176. doi:10.4049/jimmunol.1900795
- Cui X, Zhou M, Tan J, et al. Design, synthesis, and bioactive screen in vitro of cyclohexyl (E)-4-(hydroxyimino)-4-phenylbutanoates and their ethers for anti-hepatitis B virus agents. *Molecules.* 2019;24(11):2063. doi:10.3390/molecules24112063

- Wang A, Wu S, Tao Z, et al. Design, synthesis, and anti-HBV activity of new bis (l-amino acid) ester tenofovir prodrugs. *ACS Med Chem Lett.* 2019;10(6):991–995. doi:10.1021/acsmchemlett.9b00184
- Hu J, Cheng J, Tang L, et al. Virological basis for the cure of chronic hepatitis B. *ACS Infect Dis.* 2019;5(5):659–674. doi:10.1021/acsinfectdis.8b00081
- Zhang Y, Chen B, Wang L, et al. HBsAg seroclearance or seroconversion induced by peg-interferon alpha and lamivudine or adefovir combination therapy in chronic hepatitis B treatment: a meta-analysis and systematic review. *Rev Esp Enferm Dig.* 2016;108:263–270. doi:10.17235/reed.2016.3995/2015
- Caviglia GP, Abate ML, Pellicano R, Smedile A. Chronic hepatitis B therapy: available drugs and treatment guidelines. *Minerva Gastroenterol Dietol.* 2015;61:61–70.
- Tang L, Zhao Q, Wu S, Cheng J, Chang J, Guo JT. The current status and future directions of hepatitis B antiviral drug discovery. *Expert Opin Drug Discov.* 2017;12(1):5–15. doi:10.1080/17460441.2017.1255195
- Testoni B, Durantel D, Zoulim F. Novel targets for hepatitis B virus therapy. *Liver Int.* 2017;37:33–39. doi:10.1111/liv.13307
- Harris RS, Liddament MT. Retroviral restriction by APOBEC proteins. *Nat Rev Immunol.* 2004;4(11):868–877. doi:10.1038/nri1489
- Suspène R, Guétard D, Henry M, Sommer P, Wain-Hobson S, Vartanian JP. Extensive editing of both hepatitis B virus DNA strands by APOBEC3 cytidine deaminases in vitro and in vivo. *Proc Natl Acad Sci U S A.* 2005;102(23):8321–8326. doi:10.1073/pnas.0408223102
- Noguchi C, Ishino H, Tsuge M, et al. G to A hypermutation of hepatitis B virus. *Hepatology.* 2005;41(3):626–633. doi:10.1002/hep.20580
- Nguyen DH, Gummuru S, Hu J. Deamination-independent inhibition of hepatitis B virus reverse transcription by APOBEC3G. *J Virol.* 2007;81(9):4465–4472. doi:10.1128/JVI.02510-06
- Nguyen DH, Hu J. Reverse transcriptase- and RNA packaging signal-dependent incorporation of APOBEC3G into hepatitis B virus nucleocapsids. *J Virol.* 2008;82(14):6852–6861. doi:10.1128/JVI.00465-08
- Mao R, Zhang J, Jiang D, et al. Indoleamine 2,3-dioxygenase mediates the antiviral effect of gamma interferon against hepatitis B virus in human hepatocyte-derived cells. *J Virol.* 2011;85(2):1048–1057. doi:10.1128/JVI.01998-10
- Cen S, Peng ZG, Li XY, et al. Small molecular compounds inhibit HIV-1 replication through specifically stabilizing APOBEC3G. *J Biol Chem.* 2010;285(22):16546–16552. doi:10.1074/jbc.M109.085308
- Peng ZG, Zhao ZY, Li YP, et al. Host apolipoprotein B messenger RNA-editing enzyme catalytic polypeptide-like 3G is an innate defensive factor and drug target against hepatitis C virus. *Hepatology.* 2011;53(4):1080–1089. doi:10.1002/hep.24160
- Wang H, Zhong M, Li Y, et al. APOBEC3G is a restriction factor of EV71 and mediator of IMB-Z antiviral activity. *Antiviral Res.* 2019;165:23–33. doi:10.1016/j.antiviral.2019.03.005
- Wang X, Ao Z, Danappa Jayappa K, Shi B, Kobinger G, Yao X. R88-APOBEC3Gm inhibits the replication of both drug-resistant strains of HIV-1 and viruses induced from latently infected cells. *Mol Ther Nucleic Acids.* 2014;3:e151. doi:10.1038/mtna.2014.2
- Han YX, Xue R, Zhao W, et al. Antiviral therapeutic efficacy of foscarnet in hepatitis B virus infection. *Antiviral Res.* 2005;68(3):147–153. doi:10.1016/j.antiviral.2005.09.002
- Gao LM, Han YX, Wang YP, et al. Design and synthesis of oxymatrine analogues overcoming drug resistance in hepatitis B virus through targeting host heat stress cognate 70. *J Med Chem.* 2011;54(3):869–876. doi:10.1021/jm101325h
- Huang SX, Mou JF, Luo Q, et al. Anti-hepatitis B virus activity of esculetin from *Microsorium fortunei* in vitro and in vivo. *Molecules.* 2019;24(19):3475. doi:10.3390/molecules24193475
- Zhao D, Wang X, Lou G, et al. APOBEC3G directly binds Hepatitis B virus core protein in cell and cell free systems. *Virus Res.* 2010;151(2):213–219. doi:10.1016/j.virusres.2010.05.009

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