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# Synthesis, structure–activity relationship and biological evaluation of novel N-substituted matrinic acid derivatives as host heat-stress cognate 70 (Hsc70) down-regulators

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ABSTRACT

Oxymatrine (**1**) is a natural anti-hepatitis B virus (HBV) drug that down-regulates host heat-stress cognate 70 (Hsc70) expression through a mechanism different from that of nucleosides. Taking Hsc70 as a target against HBV, 26 novel N-substituted matrinic acid analogs were designed, synthesized and evaluated for their regulation of Hsc70 mRNA expression with **1** as the lead. The SAR analysis revealed that (i) the carboxyl group at the 11-position was required for activity; (ii) introducing of a substituent on the nitrogen atom at the 12-position of **3**, especially substituted benzyl, might significantly improve the activity. Among these analogs, compound **9p** possessing *N-p*-methoxylbenzyl afforded an increased anti-HBV effect in comparison with **1**. We consider **9p** a promising anti-HBV candidate.



Oxymatrine (**1**, Fig. 1), a natural drug extracted from the plant Kushen (*Sophora japonica*), has been used to treat hepatitis B patients in China for decades with a confirmed safety.[1] Its mode of action against hepatitis B virus (HBV) is distinctly different from that of nucleosides currently used in clinic such as lamivudine (3TC) and adefovir.[2–5] Recently, we have identified host heat-stress cognate 70 (Hsc70) to be a key target of compound **1**.[6] Compound **1** significantly down-regulates host Hsc70 mRNA expression at the post transcriptional level through destabilizing Hsc70 mRNA, and then inhibits the replication of HBV and exhibits anti-HBV efficacy. As this target is not a viral enzyme, antiviral agents working through this mechanism not only inhibit the replication of wild-type HBV but also the drug-resistant mutants.[6,7] In addition, Hsc70 gene knockout showed no abnormality in mice, indicating a good safety after inhibition of Hsc70.[8] Therefore, targeting host Hsc70 is a novel and effective approach to discover highly potent and safe anti-HBV drug candidates overcoming drug-resistance.[6b]

The structure–activity relationship (SAR) analysis for down-regulation in Hsc70 was initiated with modifications of the substituents at the 1-, 13- and 14-positions with **1** as the lead.[6b] The primary SAR revealed that the oxygen atom at the 1-position is not essential for the activity. In exploring the chemical mechanism of this class of compounds and searching for potent anti-HBV agents working through down-regulation of Hsc70 expression, SAR was developed by focusing our study on the function of the ring D. Thus, the ring D of **1** or matrine (**2**) was opened, and the resultant oxymatrinic acid (**3**) or matrinic acid (**4**, Fig. 1) still retained moderate activity, similar to that of its parent **1** or **2** (Table 1). It was deduced that ring D might not be required for activity. Thus, a novel class of N-substituted matrinic acid derivatives against HBV could be obtained by introducing different

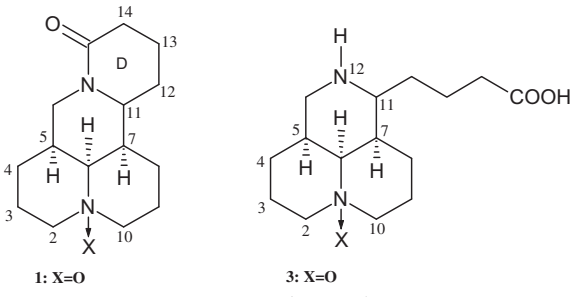


**Figure 1.** Structures of the compounds **1**–**4**.

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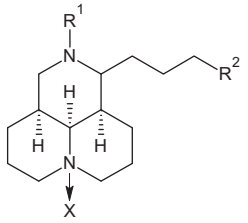
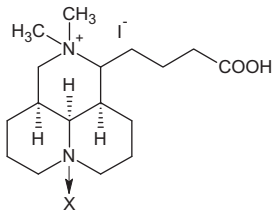
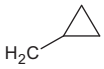
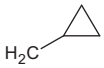
**Table 1**
Structures and down-regulating expression on Hsc70 of the N-substituted matrinic acid derivatives



**3-10**



**12 and 13**

| Compd | $R^1$ | $R^2$ | X | Hsc70 mRNA [a] |
|---|---|---|---|---|
| **1** | | | | 52.0 ± 0.10 |
| **2** | | | | 52.4 ± 0.17 |
| **3** | H | COOH | O | 45.5 ± 0.05 |
| **4** | H | COOH | / | 46.2 ± 0.05 |
| **5** | H | $CH_2OH$ | O | <10 |
| **6a** | H | $CH_2OH$ | / | 39.3 ± 0.10 |
| **6b** | $CH_2Ph$ | $CH_2OH$ | / | 25.5 ± 1.9 |
| **6c** | $CH_2PhOCH_3$-*p* | $CH_2OH$ | / | 12.2 ± 1.1 |
| **9a** | $CH_2$ $CH_3$ | COOH | / | <10 |
| **9b** | $CH_2CH_2CH_3$ | COOH | / | <10 |
| **9c** |  | COOH | / | <10 |
| **10c** |  | COOH | O | <10 |
| **9d** | $CH_2CH_2OH$ | COOH | / | 53.7 ± 0.10 |
| **9e** | CHO | COOH | / | <10 |
| **9f** | $COCH_3$ | COOH | / | 29.8 ± 0.05 |
| **9g** | $COCH_2Cl$ | COOH | / | <10 |
| **9h** | $COCH_2OH$ | COOH | / | <10 |
| **9i** | COPh | COOH | / | 33.6 ± 0.11 |
| **9j** | COCH=CHPh | COOH | / | <10 |
| **9k** | $COCH_2OPhCH_3$-*m* | COOH | / | <10 |
| **9l** | $COCH(CH_3)OPhCH_3$-*m* | COOH | / | <10 |
| **9m** | $SO_2Ph$ | COOH | / | 22.1 ± 0.08 |
| **9n** | $SO_2PhCH_3$-*p* | COOH | / | 39.8 ± 0.10 |
| **9o** | $CH_2Ph$ | COOH | / | 55.9 ± 0.13 |
| **10o** | $CH_2Ph$ | COOH | O | 58.2 ± 0.11 |
| **9p** | $CH_2PhOCH_3$-*p* | COOH | / | 72.1 ± 0.11 |
| **10p** | $CH_2PhOCH_3$-*p* | COOH | O | 69.7 ± 0.13 |
| **9q** | $CH_2PhOCH_3$-*m* | COOH | / | 55.6 ± 0.10 |
| **12** | $(CH_3)_2$ | COOH | / | 14.1 ± 0.12 |
| **13** | $(CH_3)_2$ | COOH | O | <10 |

[a] % of inhibition. Hep2.2.15 cells were cultured in the MEM medium, and incubated respectively with **1** or its analogs (100 μg/mL) for 24 h. Down-regulation of Hsc70 mRNA expression was determined with real-time RT-PCR method. The data shown were mean ± SD of three separate experiments for % of the inhibition of Hsc70 expression.

groups into the nitrogen atom at the 12-position of **3** or **4**. On the basis of this strategy, a series of N-substituted matrinic acid analogs were designed, synthesized and evaluated for their regulation of Hsc70 mRNA expression in the present study.

Twenty-eight N-substituted matrinic acid analogs were semi-synthesized as described in Scheme 1, that includes two synthetic routes (Methods A and B) with commercially available **1** or **2** as starting material. The desired products **3** and **4** were prepared via hydrolysis of **1** and **2** in aqueous KOH[9,10] and then acidification using dilute HCl with a good yield of 90% and 87%, respectively. The oxymatrinol (**5**) and matrinol (**6**) were obtained through a selective reduction of **3**, **4** or **9** with yields of 30–50%, in which $LiAlH_4$ was used as the reductive agent and THF as the solvent (Method A).[11]

The key intermediate **7** was synthesized by the carboxyl protection of **3** or **4** (Method B), in which diphenyldiazomethane was used as a protective agent and methanol and petroleum ether (60–90 °C) as the mixture solvent with good yields of 90–95%.[12] The intermediate **8** was obtained through the reaction of **7** with various RX (R = alkyl or acyl) in $CH_2Cl_2$ in the presence of anhydrous $K_2CO_3$. In this procedure, *N*,*N*-dimethyl matrinic ester (**11**) was acquired, owing to the effect of small methyl and reaction activity of $CH_3I$.[13] Finally, *m*-cresol was used as a de-protective reagent as well as solvent to transform compound **8** or **11** into desired products **9**–**10** or **12**–**13**. In the course of de-protection of **9g**, the *m*-cresol underwent condensation with intermediate **8**, and gave the product **9k**. Similarly, during the preparation of desired *N*-(2-bromopropionyl) matrinic acid, the same condensation reaction took place to get the final product **9l**.

Twenty-eight synthetic compounds were initially screened for their regulating activity on Hsc70 mRNA expression using a specific real time RT-PCR assay in human liver HepG2 cells transfected with the full genome of HBV (HepG2.2.15).[14] Structures of the analogs and their down-regulation in Hsc70 are shown in Table 1.

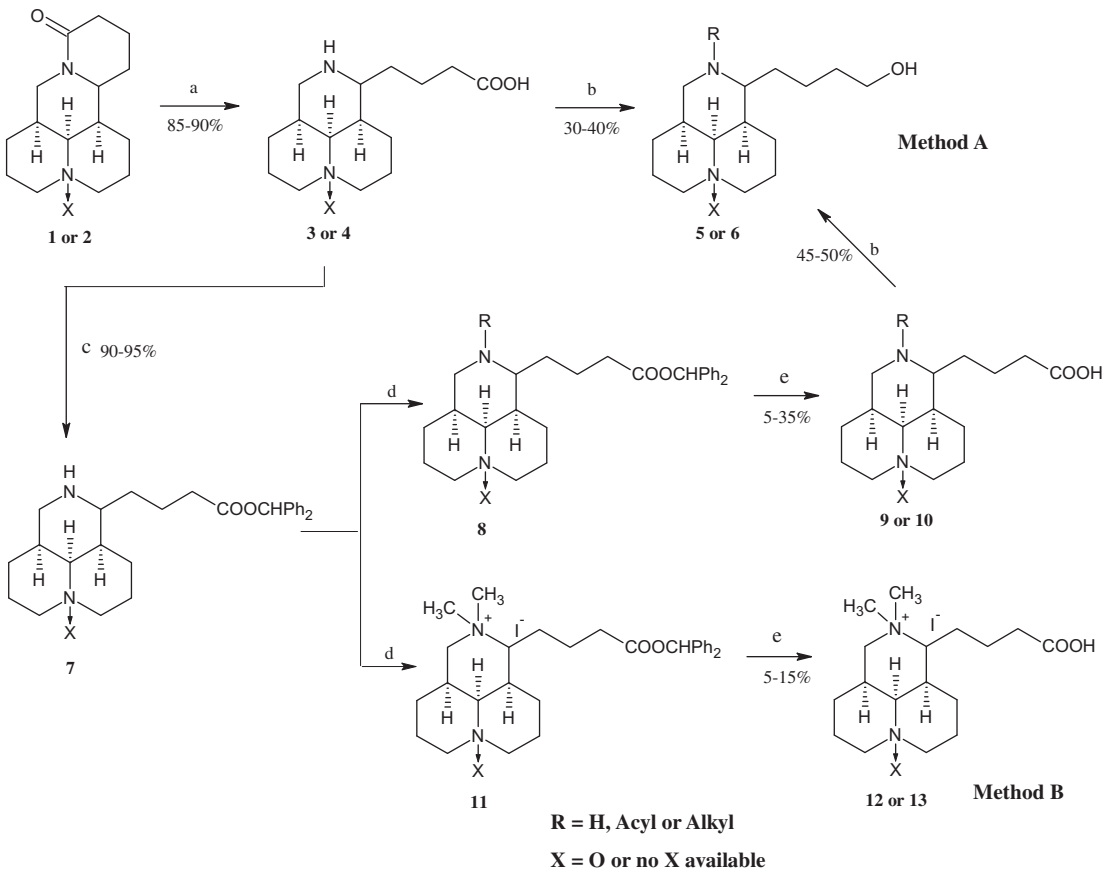
The SAR analysis was first concentrated on the variations of the substituents at position 12 of **3** or **4**. Attachment of different alkyl groups including ethyl (**9a**), *n*-propyl (**9b**), cyclopropylmethyl (**9c**) or di-methyl (**12**), all of them lost their activity on Hsc70 mRNA expression completely. Compound **9d** bearing β-hydroxyethyl at the same position afforded moderate down-regulating expression in Hsc70, similar to that of the lead **1**.

Acyl substituents including aliphatic and aromatic substitutents were done at the 12-position as well, by which eight compounds **9e**–**l** were created. The results showed that all of them lost their activity of Hsc70 down-regulation partially or completely, regardless of their size of the side chains. Similarly, sulfonyl derivatives **9m** and **9n** exhibited decreased activity of Hsc70 expression in comparison with the parent **1**. In another variation, benzyl, *p*-methoxybenzyl or *m*-methoxybenzyl substituents on the nitrogen atom (compounds **9o**–**q**) exhibited activity more or similar to the lead **1**. In particular, compound **9p**[15] bearing *p*-methoxybenzyl afforded the most potent activity in Hsc70 down-regulatory expression with about 1.3-fold increase over that of **1**. We therefore deduced that substituted benzyl at the 12-position might be helpful for the activity.

Next, SAR study was moved on the carboxyl group of compound **3** or **4**. The carboxylic acid of compounds **3**–**4**, **9o** and **9p** was reduced to alcohol, the resultant compounds **5** and **6a**–**c** exhibited a decreased activity on Hsc70 expression in comparison with its parent, respectively. Similarly, carboxyl was converted into ester or amide, respectively, and the resultant products almost lost their effect of down-regulating in Hsc70 (data not shown). It appeared that the carboxyl might play an important role in binding to speculated biological targets.

In addition, the SAR analysis for the oxygen atom at the 1-position was carried out as well. Compounds **5**, **10c** and **13** with oxygen atom at the 1-position had no activity similar to that of their parents **6a**, **9c** and **12**, respectively. Similarly, the oxygen atom was introduced into the nitrogen atom of compound **4**, **9o** or **9p**, respectively, the resultant **3**, **10o** and **10p** also retained their activity on Hsc70 expression. It is deduced that the oxygen atom at the 1-position is not an essential element in keeping compounds with potency of Hsc70 down-regulation. The results are basically consistent with our previous SAR analysis.[6b]

Five compounds **3, 4, 9n, 9o** and **9p** with moderate down-regulation in Hsc70 mRNA activity were further tested their effect against HBV in HepG2.2.15 cells with a specific real-time PCR assay. As shown in Figure 2, their inhibition rate of intracellular HBV DNA replication was basically consistent with activity in Hsc70 down-regulation. Among these analogs, compound **9p** showed the most potent anti-HBV effect, higher than **1** (77% vs 56%). In this test, 3TC was used as a positive reference; therefore,




**Scheme 1.** Synthesis of the N-substituted matrinic acid derivatives. Reagents and conditions for the chemical synthesis: (a) $KOH/H_2O$, reflux, 9 h; then 3 N HCl; (b) $LiAlH_4$, THF, reflux, 3 h; (c) diphenyldiazomethane, MeOH/petroleum ether (boiling range 60–90 °C), overnight; (d) RX, $K_2CO_3/CH_2Cl_2$, rt, 1–24 h; (e) *m*-cresol, 80 °C, 8–9 h.

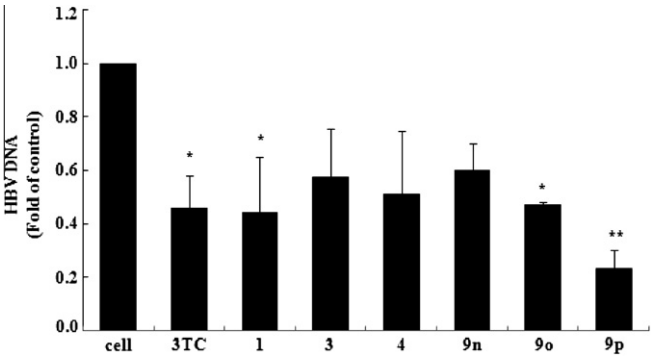



**Figure 2.** Anti-HBV activity in compounds **1**, **3**, **4**, **9o**, **9p**, **9q** and 3TC in vitro. HepG2.2.15 cells were untreated or treated with study compounds (100 μg/mL) or 3TC (30 μg/mL) as indicated for 24 h. *$p$ <0.05 as compared to that of untreated control; **$p$ <0.01 as compared to that of untreated control.

the figure also hints that the anti-HBV activity of compound **9p** is comparable to that of 3TC.

After overall evaluation, compound **9p** showed the best activity of down-regulating Hsc70 mRNA expression. Therefore, it was selected to measure down-regulating Hsc70 expression by protein level in HepG2.2.15 cells. As shown in Figure 3, compound **9p** (100 μg/mL) showed a significant activity in down-regulating Hsc70 expression by protein level, stronger than that of the lead **1** (100 μg/mL) as well. Compound **9p** exhibited the best activity of down-regulating Hsc70 protein at the concentration of 500 μg/mL. It should be mentioned here that as decrease in protein often occurs after mRNA reduction, the 24 h result for protein reduction was not as obvious as that at mRNA level.

In conclusion, by using host Hsc70 as a drug target against HBV, 28 novel N-substituted matrinic acid derivatives were semi-synthesized and evaluated for their biological effect on Hsc70 mRNA down-regulation. The SAR analysis reveals that (i) the

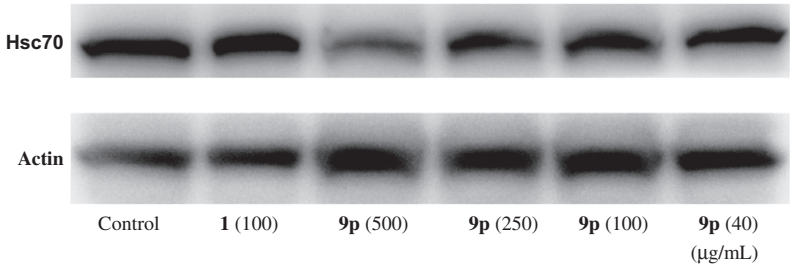



**Figure 3.** The intracellular Hsc70 protein decreased dose-dependently in the HepG2.2.15 cells untreated or treated with **1** (100 μg/mL) and **9p** (40, 100, 250 and 500 μg/mL, respectively) for 24 h.

carboxyl group is an essential element for activity; (ii) introduction of a substituent at the 12-position, especially substituted benzyl, might significantly enhance the activity. Among these analogs, compound **9p** demonstrated a down-regulatory effect on Hsc70 expression, greater than observed for **1** in HepG2.2.15 cells. We have previously reported the effect of **9p** on hepatitis C virus (HCV)-infected Huh7.5 cells, acting through the same Hsc70 mechanism.[7] Taken together, compound **9p** exhibited a broad-spectrum anti-HBV and anti-HCV effect with a mechanism different from that of nucleosides currently used in clinical patients. Also, it has a considerably good safety in vivo,[7] and thus was considered a promising drug candidate against HBV and HCV for further investigation.

## Acknowledgments

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15. *Analytical data for selected final compound* ***9p***. Mp 78.8–80.7 °C. $^1$H NMR ($CD_3OD$, 500 MHz): $\delta$ 7.39–7.40 (d, $J$ = 8.5 Hz, 2H), 6.95–6.96 (d, $J$ = 8.5 Hz, 2H), 4.66–4.69 (d, $J$ = 12.5 Hz, 1H), 3.91–3.94 (d, $J$ = 12.5 Hz, 1H), 3.74 (s, 3H), 3.36–3.41 (m, 1H), 3.12–3.19 (m, 2H), 2.99–3.02 (m, 1H), 2.82–2.86 (m, 1H), 2.61 (s, 2H), 2.36–2.45 (m, 2H), 2.16–2.22 (m, 2H), 2.03–2.06 (m, 2H), 1.57–1.91 (m, 10H), 1.49–1.51 (d, $J$ = 13.0 Hz, 1H); IR: $v$ 3394 (OH), 2945, 2873 ($CH_2$), 1715 (C=O), 1612, 1515 (C=C), 1062 (C–O) cm$^{-1}$; HRMS: calcd for $C_{23}H_{34}N_2O_3$ $(M+H)^+$ 387.2648, found 387.2646.