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Synthesis, structure–activity relationship and in vitro biological evaluation of *N*-arylethyl isoquinoline derivatives as Coxsackievirus B3 inhibitors

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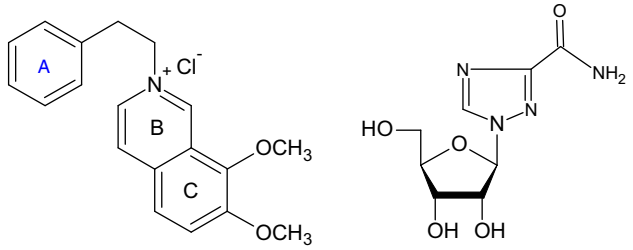
ABSTRACT

Currently, there is no approved antiviral drug for the infection caused by enteroviruses. A series of novel *N*-arylethyl isoquinoline derivatives defined with substituents on the ring A and C were designed, synthesized and evaluated in vitro for their activities against Coxsackievirus B3 (CVB3). The primary structure–activity relationship revealed that substituents on the ring A were not beneficial for the activity. Among these analogs synthesized, compound **7f** bearing a methylenedioxy at the R^4 and R^5 positions afforded an anti-CVB3 activity and a reasonable selectivity index (SI = 26.8); furthermore, **7f** exhibited a moderate activity against enterovirus 71 (EV71) with SI value of 9.0. Thus it has been selected as an anti-enteroviral lead compound for further investigation.

Enteroviruses are a genus of single-stranded (+) RNA viruses associated with several human and mammalian diseases. Enteroviruses infect human individuals worldwide. As children are considered to exhibit a relatively weak immune system, enteroviral infection in neonates could be lethal or life threatening, with high risk for morbidity and mortality. Enteroviruses are classified into polioviruses, coxsackievirus A and B, echoviruses and enteroviruses 68–71 (EV68–71).[1,2] Coxsackievirus B3 (CVB3) is an important human pathogen inducing acute and chronic viral myocarditis in children and young adults. Moreover, CVB3 is associated with the development of aseptic meningitis, hepatitis, and meningoencephalitis.[3] Enterovirus 71 (EV71) might relate to severe central nervous system diseases.[4–8] An epidemic outbreak of EV71 infection in Taiwan in 1998 caused many severe cases and 78 deaths.[9] In the spring of 2008, a major hand-foot-and-mouth disease (HFMD) outbreak caused by EV71 in China resulted in a high aggregation of fatal cases.[10]

Numerous compounds have been reported to be selective inhibitors for enteroviruses, some of which have entered into clinical trials.[11–13] Unfortunately, there is no approved antiviral drug for the treatment of acute enteroviral infections. Therefore, there is an urgent need for developing antiviral agents for enteroviral infection, including those against CVB3 and EV71.

In search for small molecule anti-CVB3 agents, the compound library established in our laboratory was screened against CVB3 using viral cytopathogenic effect (CPE) assay.[14] In the screening test, ribavirin (RBV, Fig. 1) was selected as the positive control. Although antiviral effect of ribarivin is not considered potent, the agent is relatively effective against CVB3[12] with respect to other antiviral agents. As the reference drug is currently used for CVB3 in hospitals and has a broad spectrum antiviral effect in cell culture we select this agent as a control in the cell-based test. Through the screening hit **1** (Fig. 1) was identified, because it showed inhibitory activity with IC_{50} value of 209 μM, stronger than that of RBV (IC_{50} = 910 μM). The anti-CVB3 activity and unique chemical structure of **1** provoked



1 **RBV**

Figure 1. Chemical structures of compound **1** and RBV.

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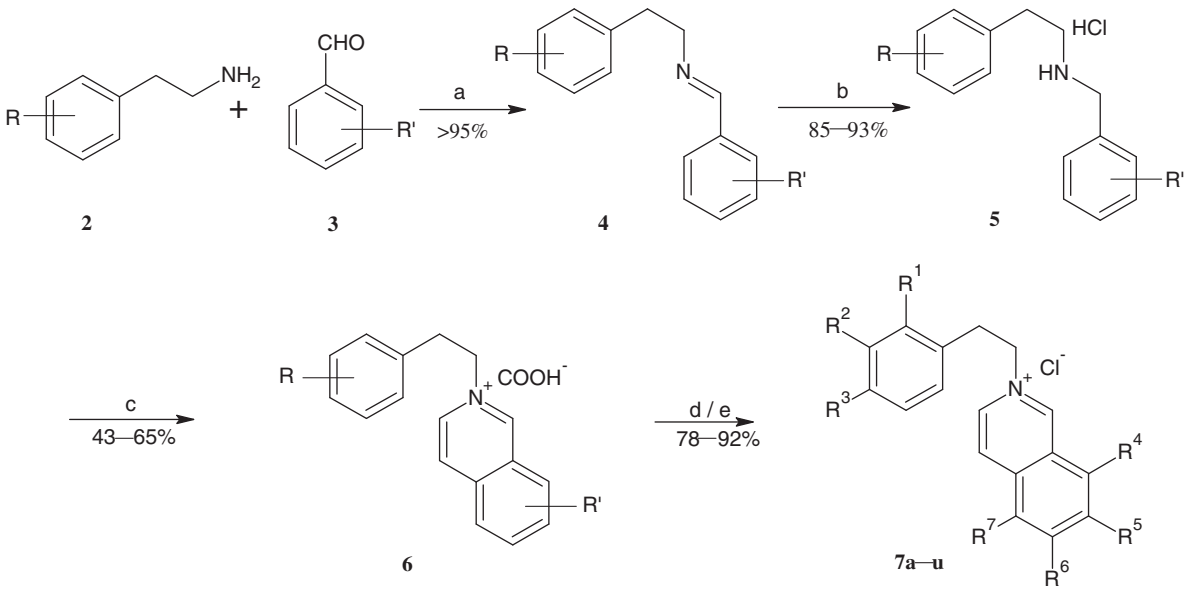


R^1, R^3 = –OCH_3, –CH_3, –Cl, –F, –H, etc;

R^2 = –Cl, –H, etc;

R^4, R^5, R^6, R^7 = –OCH_3, –OH, –OCH_2O, etc.

Scheme 1. Synthesis of the aimed compounds (**7a–u**). Reagents and conditions: (a) 100 °C, 5 h; (b) $NaBH_4$, methanol, 4 h; (c) CHO–CHO, anhydrous formic acid, $CuSO_4$, concentrated HCl, 100 °C, 6 h; (d) methanol/H_2O, CaO, 1.5 h; (e) concentrated HCl.

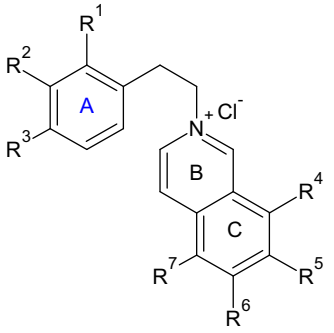
our interest to explore the structure–activity relationship (SAR) of this group of compounds, aiming for discovery of anti-CVB3 agents. Working through this strategy, a series of novel *N*-arylethyl isoquinoline derivatives were designed, synthesized and evaluated for their activities against CVB3 in vitro with **1** as the lead in the present study.

After selection of the hit compound, a novel and convenient synthetic route was developed for the desired *N*-arylethyl isoquinoline derivatives, which allows an extensive SAR analysis. Twenty-one analogs (**7a–u**) were de novo synthesized as described in Scheme 1, which demonstrates a four-step process. The method used commercially available derivatives of phenylethylamine (**2**) and benzaldehyde (**3**) as the starting materials, as described in our previous reports.[15–18] Twenty-one analogs were selectively prepared through varying substituents on the aromatic ring A and C. The activating group (–OCH_3, –OH) at the R^5 position is of assistance to the cyclization of the ring B, and the hydrogen atom or halogen such as –Cl at position R^2 on the ring A might be beneficial for the formation of the product **6** with yields of 43–65%. In the other hand, berberine analogs were obtained while an activating group exists at the R^2 position.[15–18] Then, the target compounds were purified with silica gel column chromatography using methanol/chloroform (1:20) as the eluent.

All of the study compounds were evaluated for their antiviral activity against CVB3 in African green monkey kidney cells (Vero cells), which were infected with CVB3 and measured with CPE assay. Anti-CVB3 activity was expressed with IC_{50} value and cytotoxicity with TC_{50} value. As an important therapeutic indication, the selectivity index (SI) was calculated as the ratio of TC_{50} to IC_{50}. Antiviral activity of each compound was evaluated by combining its IC_{50} with SI. Structures of the 21 *N*-arylethyl isoquinoline derivatives and their anti-CVB3 effect are shown in Tables 1 and 2, respectively.

The SAR study was mainly concentrated on the variations of substituents on the aromatic ring A and C. First, substituting –H

Table 1
Structures of the synthetic *N*-arylethyl isoquinoline derivatives (**7a–u**)



Compd	R^1	R^2	R^3	R^4	R^5	R^6	R^7
1	H	H	H	OCH_3	OCH_3	H	H
7a	H	H	F	OCH_3	OCH_3	H	H
7b	H	H	OCH_3	OCH_3	OCH_3	H	H
7c	H	H	H	OH	OCH_3	H	H
7d	H	H	OCH_3	OH	OCH_3	H	H
7e	CH_3	H	H	OH	OCH_3	H	H
7f	H	H	H	OCH_2O		H	H
7g	H	H	F	OCH_2O		H	H
7h	H	H	H	H	OCH_3	OCH_3	H
7i	H	H	F	H	OCH_3	OCH_3	H
7j	F	H	H	H	OCH_3	OCH_3	H
7k	H	Cl	H	H	OCH_3	OCH_3	H
7l	CH_3	H	H	H	OCH_3	OCH_3	H
7m	CH_3	H	H	H	OCH_3	OH	H
7n	F	H	H	H	OCH_3	OH	H
7o	H	H	H	H	OH	H	H
7p	H	Cl	H	H	OH	H	H
7q	H	H	F	H	OH	H	H
7r	CH_3	H	H	H	OH	H	H
7s	OCH_3	H	H	H	OH	H	H
7t	H	H	H	H	OCH_3	OCH_3	OCH_3
7u	H	H	OCH_3	H	OCH_3	OCH_3	OCH_3

Table 2
Anti-CVB3 activity and cytotoxicity for target compounds in Vero cells

Compd	TC_{50}[a] (μM)	IC_{50}[b] (μM)	SI[c]
1	584 ± 0.00	209 ± 13.3	2.8
7a	1383 ± 59.4	>320	—
7b	49.5 ± 4.55	>11.4	—
7c	352 ± 9.04	91.1 ± 5.74	3.9
7d	464 ± 19.8	>107	—
7e	584 ± 0.00	>337	—
7f	815 ± 37.4	30.4 ± 0.00	26.8
7g	581 ± 0.00	>335	—
7h	1012 ± 42.2	>337	—
7i	665 ± 30.0	>320	—
7j	206 ± 20.5	>53.3	—
7k	131 ± 6.23	>33.9	—
7l	970 ± 42.2	>323	—
7m	234 ± 12.5	11.2 ± 0.00	2.1
7n	333 ± 24.7	>111	—
7o	389 ± 24.7	25.0 ± 2.07	15.6
7p	200 ± 7.34	>38.6	—
7q	1098 ± 42.4	>336	—
7r	772 ± 30.0	371 ± 0.00	2.1
7s	1057 ± 42.2	>352	—
7t	2782 ± 0.00	>927	—
7u	46 ± 2.20	>10.6	—
RBV	8190 ± 0.00	910 ± 46.5	9.0

[a] Cytotoxic concentration required to inhibit Vero cell growth by 50%. Vero cells (2.5×10^4/well) were plated into a 96-well plate. A total of 24 h later, the monolayer cells were incubated in the present of various concentrations of test compounds. After 48 h of culture at 37 °C, the cells were monitored by CPE. TC_{50} value was calculated by Reed and Muench analyzes. Each experiment was repeated three times.

[b] Concentration required to inhibit CVB3 replication by 50%. Vero cells were infected with 100 median tissue culture infective dose ($100TCID_{50}$) Cox B3. Then, confluent Vero cells were cultured in the maintenance medium (MEM plus 2% FBS) and incubated, respectively with or without different concentration of test compounds. Viral CPE was observed when the viral control reached 4+ and IC_{50} was determined by the Reed and Muench analyzes. Each experiment was repeated three times.

[c] Selectivity index value equaled to TC_{50}/IC_{50}.

at the R^3 position with –F or $–OCH_3$ (**7a**–**b**) decreased the SI value, owing to the low activity or high cytotoxicity. Next, compounds (**7c**–**e**) possessing –OH at R^4 and $–OCH_3$ at the R^5 position were synthesized and analyzed. Compound **7c** with no substituent on the ring A exhibited a moderate activity with IC_{50} value of 91 μM, stronger than that of **1**. With the replacement by a methylenedioxy at the R^4 and R^5 positions (**7f**–**g**), compound **7f**[19] with the absence of substituent on the ring A exhibited a reasonable anti-CVB3 activity with SI value of 26.8, nearly threefold of that of the lead **1** (SI = 9.0). It appeared that side groups on the ring A might be a negative factor for the activity against CVB3.

Furthermore, keeping $–OCH_3$ at the R^5 position and transferring $–OCH_3$ from the R^4 to R^6 position resulted in a decrease of activity (**7h–i**, **7l**) or an increase of cytotoxicity (**7j–k**), regardless of the features of substituents on the ring A. Replacing by –OH at the R^6 position (**7m–n**) did not generate good SI values. In another variation, moving $–OCH_3$ at the R^6 position and changing the R^5 substituent from $–OCH_3$ to –OH, five compounds (**7o**–**s**) were generated. Out of the 5 compounds, compound **7o**, whose ring A was vacant, showed a promising anti-CVB3 activity with IC_{50} value of 25 μM, much stronger than that of **1**. In addition, tri-methoxy was added to positions R^4, R^5 and R^6 (**7t**–**u**), the activity of **7t** was completely abolished, and the cytotoxicity of **7u** increased significantly.

In parallel, antiviral activity against EV71 of each of the synthesized compounds was evaluated as well, using CPE method with RBV as a reference drug (Table 3).[14] Out of the 22 compounds synthesized, 4 compounds (**7f**–**g**, **7i** and **7o**) afforded a potent effect against EV71 with SI values in the range of 3.5 and 9.2 (Table 3), similar to or stronger than that of RBV (SI = 3.9). In particular, compounds **7f** and **7o** showed potential activity against either CVB3 or EV71, suggesting a broad-spectrum activity against enteroviruses by the two compounds.

Table 3
Antiviral activity against EV71 and cytotoxocity of the key compounds

Compd	TC_{50}[a] (μM)	IC_{50}[b] (μM)	SI[c]
7f	820 ± 28.7	91.1 ± 5.02	9.0
7g	420 ± 25.9	45.8 ± 3.13	9.2
7i	864 ± 33.0	107 ± 5.35	8.1
7o	534 ± 16.5	152 ± 6.64	3.5
RBV	8190 ± 0.00	2119 ± 44.1	3.9

[a] Cytotoxic concentration required to inhibit Vero cell growth by 50%. Vero cells (2.5×10^4/well) were plated into a 96-well plate. A total of 24 h later, the monolayer cells were incubated in the present of various concentrations of test compounds. After 48 h of culture at 37 °C, the cells were monitored by CPE. TC_{50} value was calculated by Reed and Muench analyzes. Each experiment was repeated three times.

[b] Concentration required to inhibit CVB3 replication by 50%. Vero cells were infected with 100 median tissue culture infective dose ($100TCID_{50}$) Cox B3. Then, confluent Vero cells were cultured in the maintenance medium (MEM plus 2% FBS) and incubated, respectively with or without different concentration of test compounds. Viral CPE was observed when the viral control reached 4+ and IC_{50} was determined by the Reed and Muench analyzes. Each experiment was repeated three times.

[c] Selectivity index value equaled to TC_{50}/IC_{50}.

In conclusion, 21 novel *N*-arylethyl isoquinoline derivatives defined with modifications on the ring A or C were designed, synthesized and evaluated for anti-CVB3 and anti-EV71 activities. The primary SAR for anti-CVB3 indicated that substituents on the ring A might reduce the antiviral activity. Among these analogs, compounds **7f** and **7o** exhibited reasonable activity against CVB3 with SI value of 26.8 and 15.6, respectively, stronger than that of RBV. In addition, compound **7f** showed moderate activity against EV71 with SI value of 9.0, higher than that of RBV. Thus, **7f** has been selected in our laboratory as a promising anti-enteroviral candidate for further investigation. The antiviral results and SAR of the *N*-arylethyl isoquinoline derivatives are of interest for further chemical modifications.

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References and notes

1. Santti, J.; Harvala, H.; Kinnunen, L.; Hyypiä, T. *J. Gen. Virol.* **2000**, *81*, 1361.
2. Kim, K. S.; Hufnagel, G.; Chapman, N. M.; Tracy, S. *Rev. Med. Virol.* **2001**, *11*, 355.
3. (a) Rotbart, H. A. *Antiviral Res.* **2002**, *53*, 83; (b) Mason, J. W. *Cardiovasc. Res.* **2003**, *60*, 5; (c) Marchant, D.; Si, X.; Luo, H.; McManus, B.; Yang, D. *Curr. Top. Microbiol. Immunol.* **2008**, *323*, 177.
4. Ooi, M. H.; Wong, S. C.; Lewthwaite, P.; Cardosa, M. J.; Solomon, T. *Lancet Neurol.* **2010**, *9*, 1097.
5. Solomon, T.; Lewthwaite, P.; Perera, D.; Cardosa, M. J.; McMinn, P.; Ooi, M. H. *Lancet Infect. Dis.* **2010**, *10*, 778.
6. Lee, T. C.; Guo, H. R.; Su, H. J.; Yang, Y. C.; Chang, H. L.; Chen, K. T. *Pediatr. Infect. Dis. J.* **2009**, *28*, 904.
7. Wong, S. S.; Yip, C. C.; Lau, S. K.; Yuen, K. Y. *Epidemiol. Infect.* **2010**, *138*, 1071.
8. Hsiung, G. D.; Wang, J. R. *J. Microbiol. Immunol. Infect.* **2000**, *33*, 1.
9. Ho, M.; Chen, E. R.; Hsu, K. H.; Twu, S. J.; Chen, K. T.; Tsai, S. F.; Wang, J. R.; Shih, S. R. *N. Eng. J. Med.* **1999**, *341*, 929.
10. Zhang, Y.; Zhu, Z.; Yang, W.; Ren, J.; Tan, X.; Wang, Y.; Mao, N.; Xu, S.; Zhu, S.; Cui, A.; Zhang, Y.; Yan, D.; Li, Q.; Dong, X.; Zhang, J.; Zhao, Y.; Wan, J.; Feng, Z.; Sun, J.; Wang, S.; Li, D.; Xu, W. *Virol. J.* **2010**, *7*, 94.
11. Aguado, L.; Thibaut, H. J.; Priego, E. M.; Jimeno, M. L.; Camarasa, M. J.; Neyts, J.; Pérez-Pérez, M. J. *J. Med. Chem.* **2010**, *53*, 316.
12. Ji, X. Y.; Zhong, Z. J.; Xue, S. T.; Meng, S.; He, W. Y.; Gao, R. M.; Li, Y. H.; Li, Z. R. *Chem. Pharm. Bull. (Tokyo)* **2010**, *58*, 1436.
13. Barnard, D. L. *Curr. Pharm. Des.* **2006**, *12*, 1379.
14. Cotarelo, M.; Catalán, P.; Sánchez-Carrillo, C.; Menasalvas, A.; Cercenado, E.; Tenorio, A.; Bouza, E. *J. Antimicrob. Chemother.* **1999**, *44*, 705.

15. Wang, Y. X.; Wang, Y. P.; Zhang, H.; Kong, W. J.; Li, Y. H.; Liu, F.; Gao, R. M.; Liu, T.; Jiang, J. D.; Song, D. Q. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6004.
16. Yang, P.; Song, D. Q.; Li, Y. H.; Kong, W. J.; Wang, Y. X.; Gao, L. M.; Liu, S. Y.; Cao, R. Q.; Jiang, J. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4675.
17. Li, Y. H.; Yang, P.; Kong, W. J.; Wang, Y. X.; Hu, C. Q.; Zuo, Z. Y.; Wang, Y. M.; Gao, H.; Gao, L. M.; Feng, Y. C.; Du, N. N.; Liu, Y.; Song, D. Q.; Jiang, J. D. *J. Med. Chem.* **2009**, *52*, 492.
18. Wang, Y. X.; Wang, L.; Xu, Y. N.; Li, Y. H.; Jiang, J. D.; Si, S. Y.; Li, Y. B.; Ren, G.; Shan, Y. Q.; Hong, B.; Song, D. Q. *Eur. J. Med. Chem.* **2011**, *46*, 1066.
19. 8-(2-Phenylethyl)[1,3]dioxolo[4,5-*h*]isoquinolin-8-ium chloride (**7f**): Yield: 52%. Melt point: 276–278 °C (dec); White solid; IR (KBr): 3446, 3021, 2959, 1612, 1501, 1459, 1293, 1265, 1198, 1176, 1017, 915; ^{1}H NMR (DMSO-d_6, ppm): δ 9.57 (s, 1H, 8-CH), 8.59 (d, 1H, J = 6.8 Hz, Ph), 8.25 (d, 1H, J = 6.8 Hz, Ph), 7.69 (s, 1H, Ph), 7.68 (s, 1H, Ph), 7.26–7.20 (m, 5H, Ph), 6.42 (s, 2H, CH_2), 4.87 (t, 2H, J = 7.2 Hz, CH_2), 3.31 (t, 2H, J = 7.6 Hz, CH_2); ^{13}C NMR (DMSO-d_6, ppm): 156.2, 151.2, 145.5, 137.4, 136.3, 134.1, 128.8, 128.6, 127.0, 125.2, 123.7, 104.11, 104.07, 103.0, 60.8, 36.3; HRMS-ESI calcd for $C_{18}H_{16}NO_2Cl$: 278.11810 $[M-Cl]^+$, found 278.11898.