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Novel *N*-Benzenesulfonyl Sophocarpinol Derivatives as Coxsackie B Virus Inhibitors

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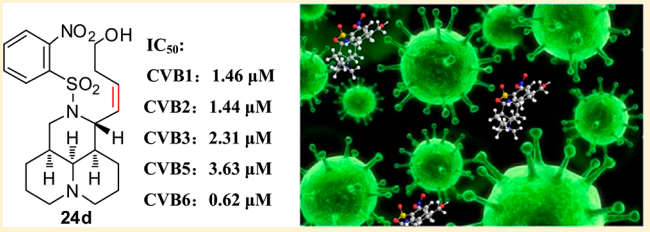
Supporting Information

ABSTRACT: Novel *N*-benzenesulfonyl sophocarpinic acid/ester and sophocarpinol derivatives were synthesized and evaluated for their antienteroviral activities against coxsackievirus type B3 (CVB3) from sophocarpine (**1**), a natural medicine isolated from Chinese herb. Structure−activity relationship (SAR) analysis revealed that the double bond and its geometrical configuration and position at the C-11 attachment did not greatly affect the potency. Among these derivatives, sophocarpinol **24d** exerted the promising activities against not only CVB3 but also CVB1, CVB2, CVB5, and CVB6 with IC_{50} ranging from 0.62 to 3.63 μM (SI from 46 to 275), indicating a broad-spectrum antienteroviral characteristic. The SAR results provided the powerful information for further strategic optimization and development of a novel scaffold of broad-spectrum antiviral candidates against enteroviruses.



KEYWORDS: *Sophocarpine, sophocarpinol, structure−activity relationship, enteroviruse, coxsakievirus type B3*

Enteroviruses are a genus of single-stranded (+) RNA viruses associated with many human diseases.[1,2] Among the enteroviruses, coxsackie B viruses (CVB) are important human pathogens causing pleurodynia, myocarditis, hepatitis, and so on.[3,4] Coxsackievirus type B3 (CVB3) is an important pathogen that induces acute and chronic viral myocarditis in children and young adults, and eventually leads to cardiomyopathy.[5,6] Besides heart infections, CVB3 causes chronic inflammatory diseases of the pancreas and central nervous system as well.[7,8] In recent years, the sudden epidemic infections of enteroviruses have caused great concerns of society.[9−11] However, there has been no special efficient drug approved for the treatment of the infections caused by CVB3 until now.[12] Therefore, there is an urgent need to develop broad-spectrum antiviral candidates against enteroviruses to meet an emergency of sudden infectious diseases.

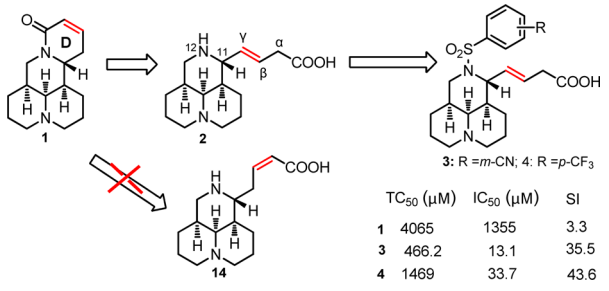
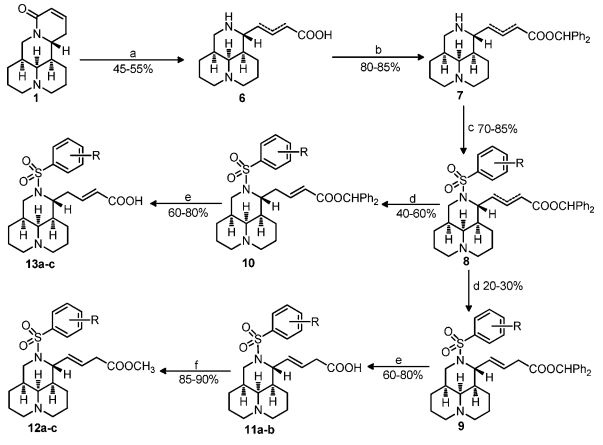


Figure 1. Chemical structures of sophocarpine (**1**); *E*-β,γ-sophocarpinic acid (**2**); *E*-β,γ-12-*N*-*m*-cyanobenzene-sulfonyl sophocarpinic acid (**3**); *E*-β,γ-12-*N*-*p*-trifluoromethy-benzenesulfonyl sophocarpinic acid (**4**); and *Z*-α,β-sophocarpinic acid (**14**).

Scheme 1[a]



[a]Reagents and conditions: (a) 5 N NaOH, reflux, 9 h, 2 N HCl, pH = 5−6; (b) diphenyldiazomethane, MeOH, r.t., 12 h; (c) $RPhSO_2Cl$, K_2CO_3, CH_2Cl_2, r.t., overnight; (d) flash column chromatography; (e) *m*-cresol, reflux, 8−9 h; (f) 2 N HCl/MeOH, reflux, 2 h.

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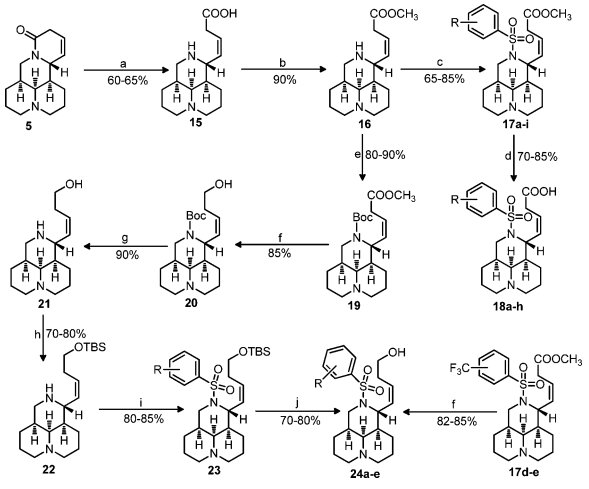
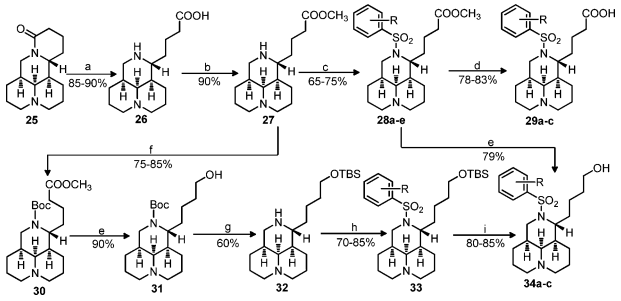
Scheme 2[a]



[a]Reagents and conditions: (a) 5 N HCl, reflux, 9 h; (b) 2 N HCl/MeOH, reflux, 2 h; (c) $RPhSO_2Cl$, K_2CO_3, CH_2Cl_2, r.t., overnight; (d) 3 N HCl, reflux, 3 h; (e) Boc_2O, K_2CO_3, CH_2Cl_2, r.t., overnight; (f) $LiAlH_4$, THF, r.t., 30 min; (g) 2 N HCl/Et_2O, 30 min; (h) TBSCl, CH_2Cl_2, imidazole, r.t., overnight; (i) $RPhSO_2Cl$, TEA, CH_2Cl_2, r.t., 4 h; (j) 2 N HCl.

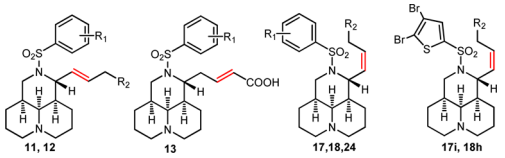
Scheme 3[a]



[a]Reagents and conditions: (a) 5 N NaOH, reflux, 9 h, 2 N HCl, pH = 5−6; (b) 2 N MeOH/HCl, reflux, 2 h; (c) $RPhSO_2Cl$, K_2CO_3, CH_2Cl_2, r.t., overnight; (d) 3 N HCl, reflux, 3 h; (e) $LiAlH_4$, THF, r.t., 30 min; (f) Boc_2O, CH_2Cl_2, K_2CO_3, r.t., overnight; (g) TBSCl, CH_2Cl_2, imidazole, r.t., overnight; (h) $RPhSO_2Cl$, TEA, 4-DMAP, CH_2Cl_2, r.t.,4 h; (i) 2 N HCl, r.t., 2 h.

Sophocarpine (**1**, Figure 1), a natural medicine extracted from Chinese herb *Sophora flavescens*, has been used to treat viral myocarditis caused by CVB3 for decades in China with an unknown mechanism of action.[13,14] The primary structure−activity relationship (SAR) investigation had been carried out with D-ring opening from compound **1**, and SAR results indicated that *E*-*β*,*γ*-sophocarpinic acid (**2**, Figure 1) with a 3-ring core was more favorable than **1** with a 4-ring scaffold. As described in Figure 1, the representative *N*-benzenesulfonyl sophocarpinic acids **3** and **4** were identified with higher potency than lead **1**,[15] and an effort to further improve the potency via modification and optimization was carried out, in order to develop a novel family of antienteroviral candidates against CVB3. In the present study, using **3**/**4** as the leads, the SAR study was mainly focused on the variations of the carboxyl group and carbon−carbon double bond located at the C-11 attachment, while 12-benzenesulfonyl moiety on the N-12 atom was maintained as a required group for CVB3.[15] Herein, we reported the synthesis, SAR analysis, and *in vitro* anti-CVB3

Table 1. Structure−Activity Relationship of Newly Synthesized Compounds against CVB3



compd	R_1	R_2	TC_{50} (μM)[a]	IC_{50} (μM)[b]	SI[c]
3	*m*-CN	COOH	466.2	13.1	35.5
4	*p*-CF_3	COOH	1469	33.7	43.6
11a	*o*-CN	COOH	466.2	22.7	20.5
11b	*o*-CF_3	COOH	393.3	11.1	15.6
12a	*m*-CN	CO_2CH_3	72.4	7.30	9.9
12b	*o*-CN	CO_2CH_3	80.3	8.92	9.0
12c	*o*-CF_3	CO_2CH_3	38.1	11.9	3.2
13a	*o*-CN	COOH	466.2	>155.4	
13b	*p*-CN	COOH	>466.2	29.9	>15.7
13c	*o*-CF_3	COOH	423.7	27.2	35.5
17a	*o*-CN	CO_2CH_3	289.2	4.21	68.7
17b	*m*-CN	CO_2CH_3	542.6	3.07	176.7
17c	*p*-CN	CO_2CH_3	292.0	8.04	36.3
17d	*m*-CF_3	CO_2CH_3	198.0	2.24	88.3
17e	*p*-CF_3	CO_2CH_3	54.7	8.17	6.7
17f	*o*-NO_2	CO_2CH_3	481.2	5.23	92.1
17g	*m*-NO_2	CO_2CH_3	111.8	9.24	12.1
17h	*p*-NO_2	CO_2CH_3	185.5	7.02	26.4
17i		CO_2CH_3	21.1	1.19	17.7
18a	*o*-CN	COOH	429.6	27. 6	15.6
18b	*m*-CN	COOH	744.8	17.4	42.7
18c	*p*-CN	COOH	744.8	52.4	14.2
18d	*p*-CF_3	COOH	272.4	18.6	14.7
18e	*o*-NO_2	COOH	594.6	16.2	36.8
18f	*m*-NO_2	COOH	445.4	63.6	7.0
18g	*p*-NO_2	COOH	308.8	49.8	6.2
18h		COOH	191.2	53.1	3.6
24a	*m*-CN	CH_2OH	147.7	5.47	27.0
24b	*m*-CF_3	CH_2OH	93.5	15.0	6.2
24c	*p*-CF_3	CH_2OH	93.5	4.17	22.4
24d	*o*-NO_2	CH_2OH	353.5	2.31	152.9
24e	*p*-NO_2	CH_2OH	265.4	17.03	15.6
RBV			8197	694.6	11.8

[a]Cytotoxic concentration required to inhibit Vero cell growth by 50%. [b]Concentration required to inhibit CVB3 growth by 50%. [c]Selectivity index: TC_{50}/IC_{50}.

evaluation of a series of novel *N*-benzenesulfonyl sophocarpinic acid/ester and sophocarpinol derivatives.

Forty-one target compounds were prepared with commercially available **1**, lehmannine (**5**), or matrine (**25**) as the starting material as described in Schemes 1−3, respectively. The D-ring in compound **1** was opened in alkaline conditions to produce an isomer mixture of *E*-sophocarpinic acid (**6**), and diphenyldiazomethane was then chosen as the protective agent to facilitate the separation of mixture **8** into its two isomers **9** and **10** with good yields. The desired acid products in **11** and **13** series were acquired by deprotection of **9** or **10** in *m*-cresol with overall yields of 5−12% as reported previously.[15,16] The sophocarpinic esters **12a−c** were obtained via methyl esterification of **11** at refluxing temperature in 2 N HCl/CH_3OH in 85−90% yields.

As shown in Scheme 2, methanol was used as the protective agent in compound **16** so as to conveniently obtain the target

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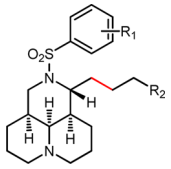
Table 2. Structure–Activity Relationship for CVB3 of Some Aimed Compounds



compd	R_1	R_2	TC_{50} (μM)[a]	IC_{50} (μM)[b]	SI[c]
28a	*m*-CN	CO_2CH_3	288.0	11.7	24.6
28b	*p*-CF_3	CO_2CH_3	73.4	2.54	28.9
28c	*o*-NO_2	CO_2CH_3	398.8	23.9	16.7
28d	*m*-NO_2	CO_2CH_3	68.9	3.51	19.7
28e	*m*-CF_3	CO_2CH_3	193.3	1.98	92.4
29a	*m*-CN	COOH	427.8	82.3	5.2
29b	*p*-CF_3	COOH	391.7	75.4	5.2
29c	*o*-NO_2	COOH	410.3	78.9	5.2
34a	*m*-CN	CH_2OH	147.0	11.3	13.0
34b	*p*-CF_3	CH_2OH	232.6	6.55	35.5
34c	*o*-NO_2	CH_2OH	243.9	35.7	6.8
RBV			8197	694.6	11.8

[a]Cytotoxic concentration required to inhibit Vero cell growth by 50%. [b]Concentration required to inhibit CVB3 growth by 50%. [c]Selectivity index: TC_{50}/IC_{50}.

products **17** with high yields as previously reported.[17] Sophocarpinols **24b–c** bearing a CF_3 substitution were directly gained through selective reduction of **17d–e** using $LiAlH_4$ as the reducing agent in THF in 82–85% yields.[17] In another synthetic route, the key intermediate **21** was acquired via *N*-*tert*-butoxycarbonyl (Boc) protection, ester reduction with $LiAlH_4$, and Boc removal in 2 N HCl from **16** with good yields. The sophocarpinols **24a** and **24d–e** possessing CN or NO_2 substitution were successfully achieved through hydroxyl protection of **21** with *tert*-butyldimethylsilyl (TBS), 12-*N*-benzenesulfonyl substitution, and deprotection of **23** in 2 N HCl with overall yields of 26–32%. Similarly, as depicted in Scheme 3, matrinic acids/esters (**28a–e** and **29a–c**) were semisynthesized from matrine with good yields as reported previously.[16,17] As mentioned above, matrinol **34b** possessing CF_3 substitution was directly acquired from **28b**; while compounds **34a** and **34c** with CN or NO_2 were obtained via a six-step sequence from compound **27** in high yields.

All the new target compounds were measured for their *in vitro* anti-CVB3 activities in African green monkey kidney (Vero) cells using viral cytopathogenic effect (CPE) assay with ribavirin (RBV) as the positive control.[18] The potency against CVB3 of each tested compound was evaluated by the combination of its IC_{50} and selectivity index (SI) value as the important therapeutic indication.

The influence of the carboxyl group at the C-11 attachment was first explored. As shown in Table 1, *E*-*β*,*γ*-sophocarpinic esters (**12a–c**) exerted potent anti-CVB3 activities with IC_{50} between 7.3 and 11.9 μM and high cytotoxic activities (TC_{50} = 38–80 μM) to give SI values 3.2–9.9. Their corresponding sophocarpinic acid (**3** and **11a–b**) with weaker activities and toxicities gave SI values ranging from 15.6 to 35.5. Then, *E*-*α*,*β*-sophocarpinic acids (**13a–c**) were generated to investigate whether the position of double bond would affect the activity. Compounds **13b–c** afforded activities comparable to their geometric isomers; while compound **13a** lost its activity, suggesting the double bond located in *β*,*γ*-position is a little more beneficial than that in *α*,*β*-position.

Next, SAR was moved on the impact of *Z*-configuration of double bond on the anti-CVB3 effect. Among *Z*-sophacarpinic ester analogues (**17a–i**), all of them displayed excellent activities with IC_{50} between 1.19 and 9.24 μM, much better than that of their corresponding sophacarpinic acids **18a–h**. Especially, compound **17b** had a promising potency with an IC_{50} of 3.07 μM and SI value of 176. Sophocarpinols **24a–e** showed good potency with IC_{50} below 17.0 μM, particularly, compound **24d** exhibited an excellent potency with an IC_{50} of 2.31 (SI = 153), suggesting that the *o*-NO_2 substituent might be helpful for the improved activity. The SAR results indicated that *E*- or *Z*-configuration of the double-bond was not an important factor for potency. Then, the double-bond was removed, and a couple of matrinic acids/esters (**28a–e** and **29a–c**) and matrinols (**34a–c**) were constructed. As shown in Table 2, all of them displayed a favorable activity against CVB3 with SI ranging from 5.2 to 92, indicating that the double bond might not play the key role for keeping good potency.

Out of the 41 new derivatives, compounds **17b**, **24d**, and **28e** demonstrated the promising anti-CVB3 effects with SI over 92.4, and then all of them were chosen for next investigation. Their antienteroviral activities against another four coxsackievirus B subtypes, including CVB1, CVB2, CVB5, and CVB6, were carried out using RBV as the positive control. As described in Table 3, compounds **17b** and **24d** afforded potencies against the four tested CVBs with IC_{50} ranging from 0.62 to 12.6 μM (SI from 39 and 275), indicating broad-spectrum antienteroviral activities against different types of CVB. It was noteworthy that compound **24d** exhibited a promising broad-spectrum antienteroviral effect against five coxsakievirus B subtypes with average IC_{50} of 1.4 μM (average SI = 142), much better than that of RBV with average SI of 6.7, and was thus selected for further investigation.

Taken together, 41 new *N*-benzenesulfonyl sophocarpinic acid/ester and sophocarpinol derivatives were designed, synthesized, and evaluated for their antienterovirus activities against CVB3. SAR analysis revealed that (i) the double-bond and its configuration and position at the 11-attachment could

Table 3. Antienteroviral Activities against Four CVB Subtypes of Representative Compounds

	17b			**24d**			**28e**			RBV		
compd	TC_{50} (μM)[a]	IC_{50} (μM)[b]	SI[c]	TC_{50} (μM)[a]	IC_{50} (μM)[b]	SI[c]	TC_{50} (μM)[a]	IC_{50} (μM)[b]	SI[c]	TC_{50} (μM)[a]	IC_{50} (μM)[b]	SI[c]
CVB1	489.8	2.60	188.4	169.8	1.46	116.0	183.3	1.28	143.5	8196	1951	4.2
CVB2	489.8	12.6	38.9	169.8	1.44	117.2	127.1	7.61	16.7	8196	2101	3.9
CVB5	489.8	10.5	46.7	169.8	3.63	46.8	73.4			8196	910.8	9.0
CVB6	489.8	2.81	174.3	169.8	0.62	275.5	61.1	0.44	139.4	8196	1708	4.8

[a]Cytotoxic concentration required to inhibit Vero cell growth by 50%. [b]Concentration required to inhibit CVB growth by 50%. [c]Selectivity index: TC_{50}/IC_{50}.

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not greatly affect the potency; (ii) the replacement of carboxyl with ester or alcohol might significantly improve the activity against CVB3. Among them, sophocarpinol **24d** exhibited the highest potency against CVB3 as well as CVB1, CVB2, CVB5, and CVB6, indicating a broad-spectrum antienteroviral feature. The SAR results provided the powerful information on further strategic modification and optimization. Overall, *N*-benzenesulfonyl matrinic acid derivatives, as a new series of compounds, offer an attractive and promising starting point for further optimization and development of a novel scaffold of broad-spectrum antiviral agents against enteroviruses.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedure, analytical data, antiviral assays, and cytotoxicity assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

§S.T. and L.K contributed equally to this work.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

CVB, coxsackievirus B; SAR, structure−activity relationship; THF, tetrahydrofuran; TLC, thin layer chromatography; TBS, *tert*-butyldimethylsilyl; Boc_2O, di-*tert*-butyl pyrocarbonate; TEA, triethylamine; RBV, ribavirin; SI, selective index; Vero cells, African green monkey kidney cells; CPE, viral cytopathogenic effect

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