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Synthetic analogs of indole-containing natural products as inhibitors of sortase A and isocitrate lyase

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ABSTRACT

Guided by the inhibitory activities of indole-containing natural products against isocitrate lyase (ICL) from *Candida albicans* and sortase A (SrtA) from *Staphylococcus aureus*, a series of compounds structurally analogous to natural products were synthesized. Eight SrtA inhibitors and an ICL inhibitor having higher activities than the natural products were discovered by screening the enzyme inhibitory activities of synthesized compounds. Among the SrtA inhibitors discovered, six exhibited higher activities than *p*-hydroxymercuribenzoic acid, which suggests that these compounds have great potential as alternative antibacterial agents.

Natural products have been regarded as a major source of pharmaceutical leads and therapeutic agents.[1] They are mostly secondary metabolites obtained by the action of various enzymes, which implies that they have great potential of efficiently interacting with enzymes. Nevertheless, natural products have attracted little research interest in recent years due to the limited quantities that can be obtained from nature, lengthy and low-yielding syntheses, and non-druggable physicochemical properties.[2] To overcome these disadvantages, efforts have been made to construct chemical libraries based on natural products.[3] Compounds bearing the structural features of natural products as well as druggable properties have been synthesized and constituted into chemical libraries. In continuation of our researches on the discovery of inhibitors of enzymes related to antimicrobial activities,[4] we have built a small chemical library with indole-type natural product scaffolds and examined the enzyme inhibitory activities of the library compounds. Sortase A (SrtA) and isocitrate lyase (ICL) were selected as target enzymes since they play crucial roles in the survival or virulence of various pathogenic bacteria and fungi.[5–8]

SrtA is an enzyme that catalyzes the covalent attachment of surface proteins to the peptidoglycan cell layer in Gram-positive bacteria such as *Staphylococcus aureus*.[5] Since surface proteins promote interactions between the invading pathogen and animal tissues, thereby providing strategies for bacterial escape from the host's immune response, SrtA has been regarded as a promising target in the development of efficient antibacterial agent.[7] The development of SrtA inhibitors has been attempted by examining natural products or high-throughput screening of chemical libraries; however, efficient SrtA inhibitors have not been discovered yet.

ICL is an enzyme that transforms isocitrate into glyoxylate in the glyoxylate cycle. The glyoxylate cycle is a reaction sequence in which acetates are converted to succinates during the energy production and biosynthesis of cell constituents; this cycle enables bacteria and fungi to grow on acetate in a hostile environment inside the macrophage where glucose is not available.[6] It has been discovered that the microbial virulence of *Candida albicans* significantly decreased in the case of mutant strains lacking the ICL. However, limited examples of ICL inhibitors have been reported thus far, and they have mostly not been successful due to their low activities or high toxicities.[8]

In a previous work, we had reported the discovery of six known 5-hydroxyindole compounds (**2**–**7**) along with a novel compound 6-hydroxydihydro-β-carboline **1** from the tropical sponge *Hyrtios* sp. and examined their inhibitory activities against ICL from *C. albicans* (Fig. 1).[4a] Among them, bis-5-hydroxyindole containing glyoxy linker (**7**) showed promising activity level (IC$_{50}$: 29 μg/mL, 89.0 μM). Subsequently, the inhibitory activities of these compounds against SrtA from *S. aureus* were examined;[10] however, inhibitions were not observed with any of the compounds except dihydro-β-carboline **1** that exhibited moderate inhibitory activity (IC$_{50}$: 67 μg/mL, 290 μM) (Table 1). Consequently, compounds **1** and **7** were considered to be attractive scaffolds for the construction of our chemical library due to their promising levels of

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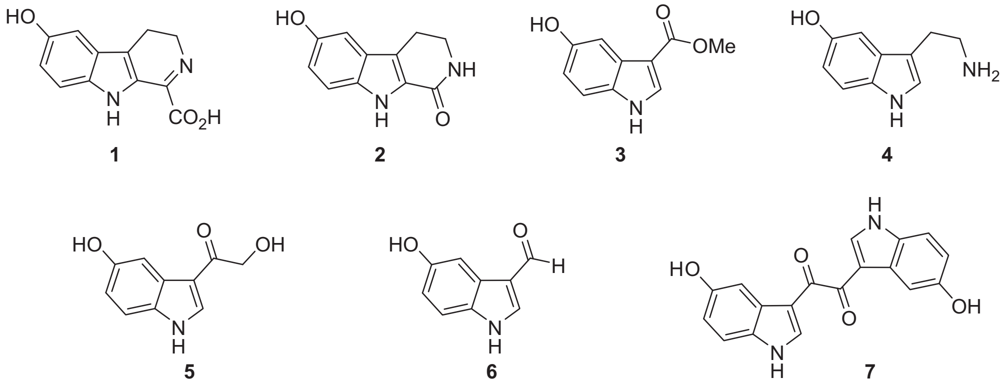


Figure 1. Compounds obtained from the tropical marine sponge *Hyrtios* sp.

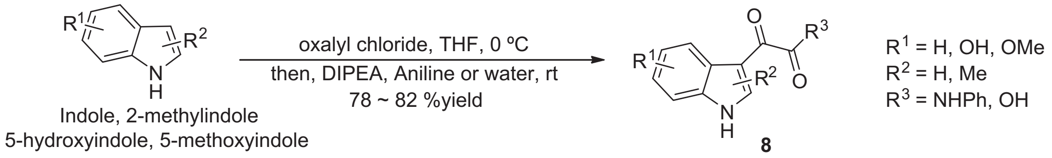
Table 1
Inhibitory activities of natural products **1**–**7** against ICL and SrtA[a]

Entry	Compound	ICL IC_{50}[b] (μg/mL, μM)	SrtA IC_{50} (μg/mL, μM)
1	**1**	87 (380)	67 (290)
2	**2**	>100	>100
3	**3**	>100	>100
4	**4**	53 (301)	>100
5	**5**	61 (318)	>100
6	**6**	40 (247)	>100
7	**7**	29 (89)	>100
8	3-NP[c]	6.0 (51)	–
9	*p*-HMB[d]	–	42 (124)

[a] Enzyme inhibitory activities were measured as described in Refs. 4,10.
[b] Previously reported data.[4a]
[c] 3-Nitropropionate, an ICL inhibitor used as a positive control.
[d] *p*-Hydroxymercuribenzoic acid, a SrtA inhibitor used as positive control.

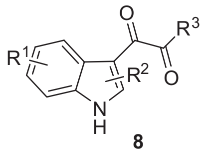
activities as well as druggable physicochemical properties.[11] In addition, easy and fast syntheses of various analogs are possible since methods for the syntheses of indoles and β-carbolines have been well studied and established.

We began our research by examining the analogs of **7**. One of the indole moieties was substituted with functionalities bearing a hydrogen bond donor (OH, NH) adjacent to the carbonyl group. Reactions between the corresponding indoles and oxalyl chloride followed by the addition of aniline or water afforded desired products (**8a**–**8h**) with high yields (78–82%) (Scheme 1).[12] Disappointingly, the synthesized analogs listed in Table 2 showed no ICL inhibitory activities; this suggests that both the indole moieties of **7** are required.[9] In the case of SrtA inhibitory activity, however, the compounds obtained from indole (**8a** and **8e**; entries 1 and 5) and 2-methylindole (**8d** and **8h**; entries 4 and 8) exhibited potent activities, while the compounds containing 5-hydroxy and 5-methoxyindole moieties exhibited no activities (entries 2, 3, 6, and 7). In particular, the SrtA inhibitory activities of compound **8d** (IC_{50}: 17 μg/mL, 61 μM) and **8e** (IC_{50}: 13 μg/mL, 60 μM) were twice as high as that of *p*-hydroxymercuribenzoic acid (*p*-HMB)[7d] (IC_{50}:



Scheme 1. Synthesis of indoleglyoxamide (**8a**–**8d**) and indoleglyoxylate (**8e** and **8f**).

Table 2
SrtA and ICL inhibitory activities of **8**[a]



8

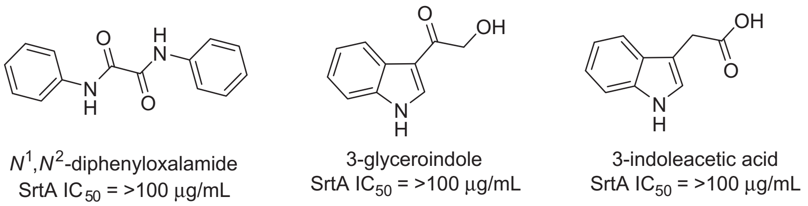
Entry	Compound	R^1	R^2	R^3	ICL IC_{50}[b] (μg/mL, μM)	SrtA IC_{50}[c] (μg/mL, μM)
1	**8a**	H	H	NHPh	>100	46 (174)
2	**8b**	OH	H	NHPh	>100	>100
3	**8c**	OMe	H	NHPh	>100	>100
4	**8d**	H	Me	NHPh	>100	17 (61)
5	**8e**	H	H	OH	>100	13 (69)
6	**8f**	OH	H	OH	>100	>100
7	**8g**	OMe	H	OH	>100	>100
8	**8h**	H	Me	OH	>100	27 (133)

[a] Enzyme inhibitory activities were measured as described in Refs. 4,10.
[b] IC_{50} of 3-nitropropionate (positive control) = 6.0 μg/mL (50 μM).
[c] IC_{50} of *p*-HMB (positive control) = 42 μg/mL (142 μM).

42 μg/mL, 124 μM), which is a known SrtA inhibitor used as a positive control (entries 4 and 5).

To gain further insights into the effects of each functional group of **8a** and **8e** on the SrtA inhibitory activity, three more analogs were examined (Fig. 2). In the case of N^1,N^2-diphenyloxalamide, wherein both the indoles of **8a** were replaced with anilines, no SrtA inhibitory activity was observed. Further, the removal of each carbonyl group (3-glyceroindole, 3-indoleacetic acid) also caused complete loss of activities. It is highly probable that both indole and α-oxocarbonyl moieties play critical roles in the interaction of compounds with SrtA.

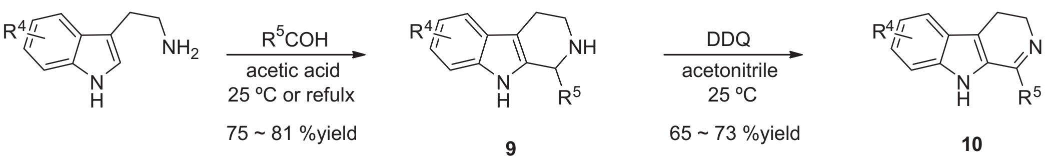
We then turned our attention to compound **1** whose analogs can be prepared easily by Pictet-Spengler reactions.[13a] Tetrahydro-β-carbolines **9** were synthesized from the corresponding tryptamines and aldehydes employing well-known procedures,[13] and dihydro-β-carbolines **10** were prepared by the oxidation of **9** (Scheme 2).[13d] In contrast to dihydro-β-carboline **1**, analogous tetrahydro-β-carboline **9b** showed no activity against both enzymes,


N^1,N^2-diphenyloxalamide
SrtA IC_{50} = >100 μg/mL

3-glyceroindole
SrtA IC_{50} = >100 μg/mL

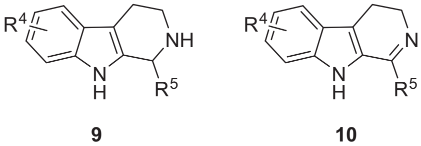
3-indoleacetic acid
SrtA IC_{50} = >100 μg/mL

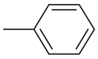
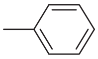
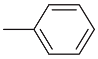
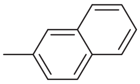
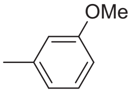
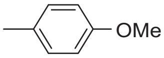
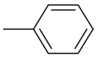
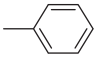
Figure 2. Loss of SrtA inhibitory activities caused by the deletion of an indole or carbonyl moieties.



Scheme 2. Syntheses of tetrahydro-β-carbolines (**9**) and dihydro-β-carbolines (**10**).

Table 3
SrtA and ICL inhibitory activities of **9** and **10**[a]



Entry	Compound	R^4	R^5	ICL IC_{50}[b] (μg/mL, μM)	SrtA IC_{50}[c] (μg/mL, μM)
1	**9a**	H–	$-CO_2H$	>100	>100
2	**9b**	OH	$-CO_2H$	>100	>100
3	**9c**	OMe	$-CO_2H$	>100	>100
4	**9d**	H	$-C_5H_{11}$	>100	>100
5	**9e**	OMe	$-C_5H_{11}$	>100	>100
6	**9f**	H		>100	>100
7	**9g**	OH		>100	28 (106)
8	**9h**	OMe		>100	32 (115)
9	**9i**	H		41 (137)	>100
10	**9j**	H	 OMe	21 (75)	>100
11	**9k**	H	 OMe	50 (180)	>100
12	**10a**	H		>100	24 (97)
13	**10b**	OMe		>100	7 (25)

[a] Enzyme inhibitory activities were measured as described in Refs. 4,10.
[b] IC_{50} of 3-nitropropionate (positive control) = 6.0 μg/mL (50 μM).
[c] IC_{50} of *p*-HMB (positive control) = 42 μg/mL (142 μM).

Table 4
Minimum inhibitory concentrations (MICs) of selected synthesized analogs against bacterial strains[a,b]

Entry	Compound	MIC (μg/mL, μM)					
		SA	BS	ST	PV	ML	EC
1	**8a**	>200	>200	>200	>200	>200	>200
2	**8d**	>200	>200	>200	>200	>200	>200
3	**8e**	>200	>200	>200	>200	>200	>200
4	**8h**	>200	>200	>200	>200	>200	>200
5	**9g**	100	100	100	100	100	>200
6	**9h**	>200	>200	>200	>200	>200	>200
7	**9i**	12.5	6.25	12.5	6.25	12.5	50
8	**9j**	6.25	12.5	12.5	12.5	12.5	50
9	**9k**	12.5	6.25	6.25	6.25	6.25	50
10	**10a**	>200	>200	>200	>200	>200	>200
11	**10b**	>200	>200	>200	>200	>200	>200
12	Ampicillin	1.56	0.78	1.56	0.78	0.78	50

[a] MIC value represents concentration giving complete inhibition relative to the negative control. See Ref. 7a for detailed procedures.

[b] SA, *Staphylococcus aureus* ATCC 6538p; BS, *Bacillus subtilis* ATCC 6633; ST, *Salmonella typhimurium* ATCC 14028; PV, *Proteus vulgaris* ATCC 3851; ML, *Micrococcus luteus* IFC 12708; EC, *Escherichia coli* ATCC 25922.

as given in Table 3. Neither of the derivatives of **9b** (**9a**, **9c**) exhibited any activities (entries 1–3). Compounds bearing a pentyl group instead of carboxylic acid (**9b** and **9e**) also showed no activities (entries 4 and 5).

When carboxylic acid groups were replaced with the phenyl ring, dramatic increases in SrtA inhibitory activities were observed in the case of **9g** (IC_{50}: 28 μg/mL, 106 μM) and **9h** (IC_{50}: 32 μg/mL, 115 μM); however, **9f** remained inactive (entries 6–8). The oxidation of **9f** and **9h** to **10a** (IC_{50}: 24 μg/mL, 97 μM) and **10b** (IC_{50}: 7 μg/mL, 25 μM), respectively, caused further increases in activities (entries 12 and 13), and the activities of these four compounds were higher than that of *p*-HMB.

Increasing the size of the phenyl ring by further substitutions resulted in significant improvements in ICL inhibitory activities (entries 9–11). In particular, compound **9j** bearing the *m*-methoxyphenyl ring exhibited higher ICL inhibitory activity (IC_{50}: 21 μg/mL, 75 μM) higher than the natural product **7** (29 μg/mL, 89 μM), which is the most potent ICL inhibitor among the natural products (Table 1, entry 7).

The effects of the obtained enzyme inhibitors on the bacterial growth were determined by employing a two fold microtiter broth dilution method against various bacterial strains (Table 4). The minimum inhibitory concentrations (MICs) were determined according to a published protocol.[7a] SrtA inhibitors (entries 1–6, entries 10–11) exhibited no growth inhibitory activity against all types of strains, except in the case of **9g** that insignificantly inhibited the growth of most cell lines (entry 5). In the case of the SrtA inhibitor, this result might suggest selective inhibition of SrtA or low toxicity of these compounds as it is known that SrtA inhibitors disrupt the pathogenesis of bacterial infections without affecting the microbial viability.[14] On the other hand, ICL inhibitors (**9i**–**9k**; entries 7–9) caused moderate to high growth inhibitions of most of the strains as expected.

In conclusion, we have prepared a series of synthesized analogs of natural products, which exhibit ICL and SrtA inhibitory activities. Among the 21 synthesized analogs, an indoleglyoxamide **8d**, an indoleglyoxylate **8e**, and four β-carbolines (**9g**, **9h**, **10a**, and **10b**) exhibited SrtA inhibitory activities higher than that of *p*-HMB. A tetrahydro-β-carboline **9j** with ICL inhibitory activities higher than those of natural products (**1**–**7**) was also discovered. It would be worthwhile to emphasize that all of the synthesized compounds were predicted to have druggable physicochemical properties like natural products **1** and **7**.[11] Further investigations into the development of efficient antibacterial agents based on these results are still in progress.

Acknowledgments

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References and notes

1. (a) Li, J. W. H.; Vederas, J. C. *Science* **2009**, *324*, 161; (b) Harvey, A. L. *Drug Discov. Today* **2008**, *13*, 894; (c) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2007**, *70*, 461.
2. Lam, K. S. *Trends Microbiol.* **2007**, *15*, 279. and references cited therein.
3. (a) John, J. E. *Drug Discov. Today* **2010**, *15*, 409; (b) Bugni, T. S.; Richard, B.; Bhoite, L.; Cimbora, D.; Harper, M. K.; Ireland, C. M. *J. Nat. Prod.* **2008**, *71*, 1095; (c) Messer, R.; Fuhrer, C. A.; Häner, R. *Curr. Opin. Chem. Biol.* **2005**, *9*, 259; (d) Boldi, A. M. *Curr. Opin. Chem. Biol.* **2004**, *8*, 281.
4. (a) Lee, H.-S.; Yoon, K.-M.; Han, Y.-R.; Lee, K.-J.; Chung, S.-C.; Kim, T.-I.; Lee, S.-H.; Shin, J.; Oh, K.-B. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1051; (b) Lee, H.-S.; Lee, T.-H.; Yang, S. H.; Shin, H. J.; Shin, J.; Oh, K.-B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 2483.
5. (a) Ton-That, H.; Schneewind, O. *J. Biol. Chem.* **1999**, *274*, 24316; (b) Mazmanian, S. K.; Liu, G.; Ton-That, H.; Schneewind, O. *Science* **1999**, *285*, 760.
6. (a) Muñoz-Elías, E. J.; McKinney, J. D. *Nat. Med.* **2005**, *11*, 638; (b) Lorez, M. C.; Fink, G. R. *Nature* **2001**, *412*, 83.
7. (a) Frankel, B. A.; Bentley, M.; Kruger, R. G.; McCafferty, D. G. *J. Am. Chem. Soc.* **2004**, *126*, 3404; (b) Kudryavtsev, K.; Bentley, M. L.; McCafferty, D. G. *Bioorg. Med. Chem.* **2009**, *17*, 2886; (c) Suree, N.; Yi, S. W.; Thieu, W.; Marohn, M.; Damoiseaux, R.; Chan, A.; Jung, M. E.; Clubb, R. T. *Bioorg. Med. Chem.* **2009**, *17*, 7174; (d) Kim, S.-H.; Shin, D.-S.; Oh, M.-N.; Chung, S.-C.; Lee, C.-S.; Oh, K.-B. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 421; (e) Park, B.-S.; Kim, J.-G.; Kim, M.-R.; Lee, S.-E.; Takeoka, G. R.; Oh, K.-B.; Kim, J.-H. *J. Agric. Food Chem.* **2005**, *53*, 9005; (f) Jang, K. H.; Chung, S.-C.; Shin, J.; Lee, S.-H.; Kim, T.-I.; Lee, H.-S.; Oh, K.-B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5366.
8. (a) Lee, H.-S.; Lee, T.-H.; Lee, J.-H.; Chae, C.-S.; Chung, S.-C.; Shin, D.-S.; Shin, J.; Oh, K.-B. *J. Agric. Food Chem.* **2007**, *55*, 6923; (b) Yang, H.-C.; Yu, J.; Oh, K.-B.; Shin, D.-S.; Cho, W.-J.; Shin, J.; Kim, S. *Arch. Pharm. Res.* **2007**, *30*, 955.
9. For the cloning, expression and purification of ICL from the genomic DNA of *C. albicans* (ATCC 10231) and the procedure for the evaluation of inhibitory activities of compounds against ICL, see: (a) Shin, D.-S.; Kim, S.; Yang, H.-C.; Oh, K.-B. *J. Microbiol. Biotechnol.* **2005**, *15*, 652; (b) Hautzel, R.; Anke, H.; Sheldric, W. S. *J. Antibiot.* **1990**, *43*, 1240.
10. For the procedure for the evaluation of inhibitory activities of compounds against SrtA, see Ref. 7d.
11. Natural products **1** and **7** show zero violations of Lipinski's rule of five and Veber rules. The physicochemical properties such as log *P*, polar surface are, number of hydrogen bond donors and acceptors, and number of rotational bonds were predicted using Molinspirations (Molinspiration Cheminformatics, Nova ulica 61, SK-900 26 Slovensky Grob, Slovak Republic. Available online at: http://www.molinspiration.com/cgi-bin/properties). For Lipinski's rule of five, see: (a) Lipinski, C. A.; Lombardo, F.; Dominy, B. W. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3; For Veber rules, see; (b) Veber, D. F.; Johnson, S. R.; Cheng, H. Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
12. (a) Thompson, M. J.; Borsenberger, V.; Louth, J. C.; Judd, K. E.; Chen, B. *J. Med. Chem.* **2009**, *52*, 7503; (b) Gitto, R.; Luca, D. L.; Ferro, S.; Citraro, R.; Sarro, G. D.; Costa, L.; Ciranna, L.; Chimirri, A. *Bioorg. Med. Chem.* **2009**, *17*, 1640.
13. (a) Cox, E. D.; Cook, J. M. *Chem. Rev.* **1995**, *95*, 1797; (b) Trujillo, J. I.; Meyers, M. J.; Anderson, D. R.; Hegde, S.; Mahoney, M. W.; Vernier, W. F.; Buchler, I. P.; Wu, K. W.; Yang, S.; Hartman, S. J.; Reitz, D. B. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4657; (c) Kawashima, Y.; Horiguchi, A.; Taguchi, M.; Tuyuki, Y.; Karasawa, Y.; Araki, H.; Hatayama, K. *Chem. Pharm. Bull.* **1995**, *43*, 783; (d) Shen, Y.-C.; Chen, C.-Y.; Hsieh, P. W.; Duh, C.-Y.; Lin, Y.-M.; Ko, C.-L. *Chem. Pharm. Bull.* **2005**, *53*, 32.
14. (a) Jonsson, I. M.; Mazmanian, S. K.; Schneewind, O.; Bremell, T.; Tarkowski, A. *Microbes Infect.* **2003**, *5*, 775; (b) Mazmanian, S. K.; Liu, G.; Lenoy, E. R.; Schneewind, O. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5510.