JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Scaffold Tailoring by a Newly Detected Pictet–Spenglerase Activity of Strictosidine Synthase: From the Common Tryptoline Skeleton to the Rare Piperazino-indole Framework

Fangrui Wu,[†,‡] Huajian Zhu,[†,‡] Lianli Sun,[†,‡] Chitra Rajendran,[⊥] Meitian Wang,[⊥] Xin Ren,[∥] Santosh Panjikar,[§] Artem Cherkasov,[∥] Hongbin Zou,*[,†] and Joachim Stöckigt*[,†]

[†]Institute of Materia Medica, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China
[⊥]Swiss Light Source at Paul Scherrer Institute, Villigen CH-5232, Switzerland
[∥]Vancouver Prostate Centre, University of British Columbia, Vancouver, British Columbia V6H 3Z6, Canada
[§]Australian Synchrotron, 800 Blackburn Road, Victoria 3168, Australia

Supporting Information

ABSTRACT: The Pictet–Spenglerase strictosidine synthase (STR1) has been recognized as a key enzyme in the biosynthesis of some 2000 indole alkaloids in plants, some with high therapeutic value. In this study, a novel function of STR1 has been detected which allows for the first time a simple enzymatic synthesis of the strictosidine analogue **3** harboring the piperazino[1,2-*a*]indole (PI) scaffold and to switch from the common tryptoline (hydrogenated carboline) to the rare PI skeleton. Insight into the reaction is provided by X-ray crystal analysis and modeling of STR1 ligand complexes. STR1 presently provides exclusively access to **3** and can act as a source to generate by chemoenzymatic approaches libraries of this novel class of alkaloids which may have new biological activities. Synthetic or natural monoterpenoid alkaloids with the PI core have not been reported before.

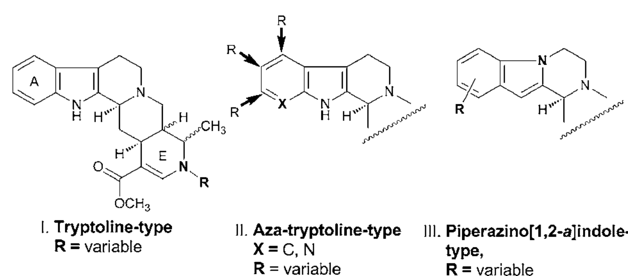
Chemical synthesis has played a leading role for many years in the preparation of both known and novel chemotypes of biologically active molecules.[1] This approach has been significantly complemented by the use of enzyme-mediated synthesis.

In the indole alkaloid field, both wild-type and rationally engineered variants of strictosidine synthase (STR1) have recently been applied effectively *in vivo*[2] and *in vitro*[3] to catalyze Pictet–Spengler reactions (PSRs),[3,4] making this enzyme an attractive subject for extensive studies of directed biosynthesis and chemoenzymatic synthesis, as outlined in a recent review.[5]

STR1 has been shown to play a key role in the biosynthesis of some 2000 structurally distinct monoterpenoid indole alkaloids,[6] some of which have high therapeutic value.[7] The reverse genetics-based isolation and cloning of STR1 cDNA from the traditional Indian and Chinese medicinal plant genus *Rauvolfia*,[8] followed by heterologous expression in up to 40 L cultures of *Escherichia coli*, can now routinely provide preparative-scale amounts of this Pictet–Spenglerase.

The X-ray crystal structure of STR1 in complex with both the natural substrate tryptamine and the product 3*α*-(*S*)-strictosidine show that in the binding pocket there is a tight arrangement of amino acids surrounding the A-ring (Scheme 1).[5] The structure helps to explain the earlier biochemical

Scheme 1. STR1-Based Chemoenzymatic Strategies toward Novel Alkaloid Structures with Tryptoline (I), Aza-tryptoline (II), and Piperazino[1,2-*a*]indole (III)



observation of a pronounced substrate specificity of this enzyme. For example, either A-ring-modified tryptamines were not accepted by STR1 or their transformation rates were <10% compared to tryptamine. Based on these observations three new chemoenzymatic strategies have been developed to generate novel alkaloids (Scheme 1):

(i) Mimicking of heteroyohimbine-type alkaloid biosynthesis leading to E-ring-modified *N*-analogous heteroyohimbines (structure **I**).
(ii) Formation and transformation of A-ring-modified strictosidines using re-engineered STR1 mutants (structure **II**).
(iii) STR1-mediated synthesis of novel strictosidines containing the piperazino-indole core instead of the tetrahydro-*β*-carboline (tryptoline) unit (structure **III**).

Here we report the detection of a new Pictet–Spenglerase activity of STR1 which allows for the first time the ability to switch enzymatically from the usually obtained tryptoline skeleton to the less frequently described piperazino[1,2-*a*]indole scaffold.

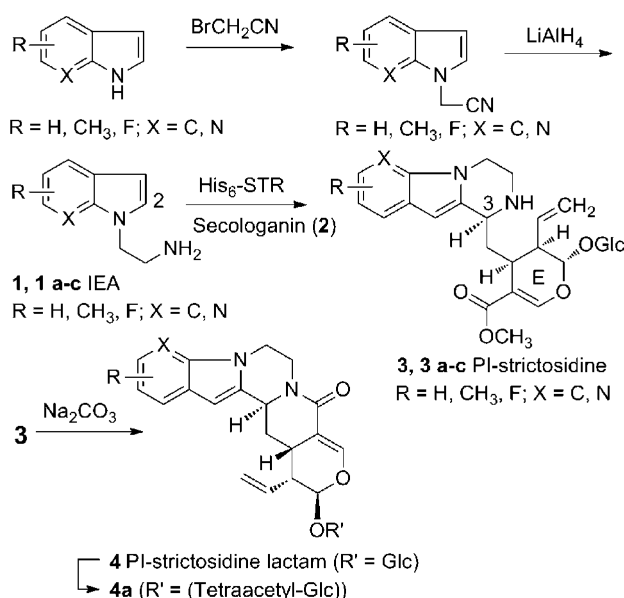
Received: December 9, 2011
Published: December 29, 2011

© 2011 American Chemical Society

In light of the rising interest in the pyrazino- and piperazino[1,2-*a*]indole systems due to their wide range of biological activities,[9] a variety of methods for their synthesis have emerged. Most synthetic strategies, especially for regio- and enantioselective synthesis, have required relatively demanding catalytic chiral or transition-metal catalysts.[10] There are no reported examples of the use of enzyme catalysis to prepare this class of compounds.

Indeed, surprisingly few naturally occurring alkaloids with a piperazine skeleton, such as the mycotoxin gliotoxin[11a] or the bis-indolalkaloid dragmacidin and its isomers, have been described.[11bc] Synthetic or natural monoterpenoid alkaloids with the piperazino[1,2-*a*]indole core, none of which have been reported before, are now available by chemoenzymatic synthesis (Scheme 2).

Scheme 2. Chemical Synthesis of 1*H*-Indole-1-ethanamine (1, IEA), Its Derivatives, and STR1-Catalyzed PI-Strictosidine (3) (R = variable)



In order to evaluate the substrate tolerance and to extend the STR1-catalyzed Pictet–Spengler reaction to more complex tryptamine derivatives, 1*H*-indole-1-ethanamine (1, IEA) was synthesized (Supporting Information (SI)-2) and tested as a possible substrate. Incubation of recombinant STR1 wild-type with the indole derivative 1 under neutral conditions in the presence of the monoterpenoid glucoside secologanin (2) resulted in excellent enzyme-dependent conversion (>95%), as shown by HPLC analysis (Figure S1A, SI). The strictosidine analogue (3) was formed in which the typical tryptoline (hydrogenated carboline) skeleton was replaced by the unusual piperazino[1,2-*a*]indole framework, representing the first example of a piperazino-monoterpenoid indole alkaloid. Spectroscopic data (SI-6,11) were in full accord with the structure of the novel piperazino[1,2-*a*]indolyl-strictosidine (3, PIS). Rigorous proof of the stereochemistry at C-3 of 3 was obtained by ^{1}H NMR analysis of the derived lactam-tetraacetate (4a).[3b,d,6a] NMR data unequivocally established the C-3α-(*S*) configuration of 3.[3b,d,6a] As in the case of the natural STR1 substrate tryptamine, the complete stereoselectivity of STR1 also delivers exclusively 3α-(*S*)-PIS (3) by condensation of 1 and 2. Mechanistically, the enzyme reaction resembles strictosidine formation with the catalytic residue Glu309 (mutant Glu309Ala is inactive), and the mutant Tyr151Phe exhibits no influence on enzyme activity.

The reaction depends entirely on active STR1 and will not proceed (a) without STR1 and (b) under either biomimetic conditions (pH 2, 6 h, 100 °C), as reported for generation of a strictosidine/3β-(*R*)-vincoside mixture,[12,13] or acidic conditions (pH 2.5) (Figure S1B–D). The use of the enzyme STR1 presently provides exclusive access to 3.

Comparison of the relative activity of STR1 for tryptamine and its analogue 1 revealed only ~26% activity for the latter. The conversion is significantly higher than for all previously tested tryptamine derivatives but well below the rate of the native substrate (100%). To address this point and to gain more insight into this reaction, we determined the X-ray crystal structure of the complex of STR1 with IEA (1) at 2.3 Å resolution (Figure 1A). The unnatural amine substrate 1 is positioned in the center of the catalytic pocket of STR1 with the primary amine group clearly coordinated with the side chain of Glu309, known to be the catalytic residue of STR1. Based on the X-ray structure, there are several structural arguments for the lower STR1 activity observed for the conversion of 1 compared to the native tryptamine. First, the distance between the amino group of 1 and Glu309 is 2.9 Å, compared to the closer distance (2.5 Å) for the hydrogen bond in the native STR1–tryptamine complex.[3a,4a] The resulting

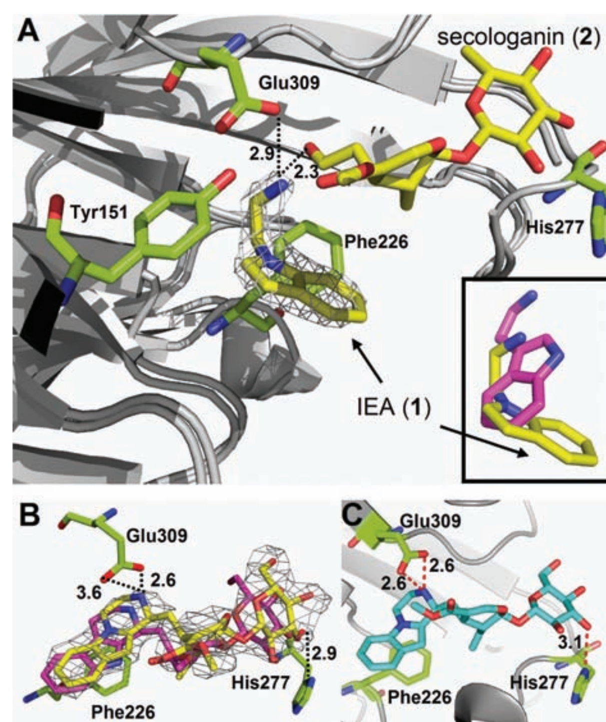


Figure 1. (A) Enlarged image of the catalytic center of the two superimposed crystal complexes of STR1 with IEA (1, PDB 3VIS) and secologanin (2, PDB 2FPC), shown with the catalytic residue Glu309. The indole part of 1 is perpendicular located between the aromatic ring of Tyr151 and Phe226. Small frame compares the conformation of 1 and native tryptamine in STR1 (PDB 2FPB) crystal complexes, respectively. (B) The crystallized PI-strictosidine-STR1 ligand complex (yellow) superimposed on the complex of STR1-strictosidine (PDB 2V91) (pink). (C) PI-strictosidine modeled into the binding pocket of STR1 (PDB 2V91).

weaker interaction might diminish the deprotonation ability of Glu309 for **1**, which likely represents the first step of the PSR.[3a,4b] Second, if the ligand complex of **1** was superimposed with the native STR1-secologanin (**2**) complex,[3a] the gap between the amine and the aldehyde group of **2** should be 2.3 Å. The actual distance in both superimposed native complexes was, however, much shorter (0.92 Å), which would favor Schiff base formation between tryptamine and **2** compared to the amine **1**.[3a] In addition, the indole part of the tryptamine analogue **1** lies nearly perpendicular to the aromatic rings of Tyr151 and Phe226, resulting therefore in a decrease in $\pi-\pi$ interactions. This arrangement would make the indole portion of **1** more flexible, in contrast to the sandwich-like structure in the STR1 complex with tryptamine, a conformation resulting in optimum orientation of the amino group for the Pictet−Spengler condensation.

The distance between carbon 2 of STR1-bound IEA (**1**) and the aldehyde carbon of secologanin (**2**), which would form the C2−C3 bond of the eventual piperazino-indole scaffold, is 5.7 Å, while the comparable distance to native tryptamine is only 1.03 Å.[3a] The proximity of both carbons supports the more efficient and "direct" formation of the natural strictosidine compared to **3**. In contrast, the 5.7 Å gap might suggest that movement of the molecule in the binding pocket must take place, because superimposition of the crystallized STR1 complexes with strictosidine compared to the new PI-strictosidine (**3**) complex revealed nearly identical conformations for both ligands (rmsd 0.74 Å) (Figure 1B). This arrangement in the binding site of STR1 was also supported by docking **3** into the STR1 X-ray structure (from PDB 2V91) using Molecular Operating Environment software followed by minimizing to an energy gradient of 0.001 kcal/(mol·Å). The close similarity between the X-ray results and modeling of **3** bound in the catalytic pocket of STR1 is illustrated in Figure 1C. All these structural interactions in the catalytic pocket most likely explain the comparatively lower activity of STR1 for the synthesis of the novel PI-strictosidine (**3**).

The enzymatic synthesis of **3** and the illustrated E-ring-substituted derivatives **3a−c** now opens the way to various chemoenzymatic strategies for the preparation of the novel class of terpenoid alkaloids harboring the piperazino[1,2-*a*]indole framework. Alkaloids of this type may present a rich future source of molecules exhibiting important pharmacological activities. STR1 became an excellent tool in order to generate libraries of both "privileged structures" and "diversity oriented" alkaloidal molecules.[14]

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, HPLC, HRMS, ^{1}H NMR data, supplemental figures and schemes, and complete ref 9c. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

zouhb@zju.edu.cn; Joesto2000@yahoo.com

Author Contributions

‡These authors contributed equally.

■ ACKNOWLEDGMENTS

This work is supported by Fonds der Chemischen Industrie (Frankfurt/Main, Germany); German Science Foundation (DFG, Bad Godesberg, Germany); The National Natural Science Foundation of China (No. 20802066); The Zhejiang Qianjiang talent plan (No. 2011R10023); and K. P. Chao High-Tech Foundation of Zhejiang University. We appreciate David Cane (Brown University, USA) for critical reading of the manuscript. We also thank the staff members of Shanghai Synchrotron Radiation Facility (SSRF) and of beamline X06DA at the Swiss Light Source (Paul Scherrer Institute).

■ REFERENCES

(1) (a) Schneider, G.; Fechner, U. *Nature Rev.* **2005**, *4*, 649−663. (b) Brown, N.; Jacoby, E. *Mini-Rev. Med. Chem.* **2006**, *6*, 1217−1229. (c) Hu, Y.; Bajorath, J. *Med. Chem. Commun.* **2010**, *1*, 339−344.

(2) (a) Runguphan, W.; O'Connor, S. E. *Nat. Chem. Biol.* **2009**, *5*, 151−153. (b) Runguphan, W.; Xudong, Q.; O'Connor, S. E. *Nature* **2010**, *468*, 461−464.

(3) (a) Ma, X. Y.; Panjikar, S.; Koepke, J.; Loris, E.; Stöckigt, J. *Plant Cell* **2006**, *18*, 907−920. (b) Loris, E. A.; Panjikar, S.; Ruppert, M.; Barleben, L.; Unger, M.; Schübel, H.; Stöckigt, J. *Chem. Biol.* **2007**, *14*, 979−985. (c) Bernhardt, P.; McCoy, E. A.; O'Connor, S. E. *Chem. Biol.* **2007**, *14*, 888−897. (d) Zou, H. B.; Zhu, H. J.; Zhang, L.; Yang, L. Q.; Yu, Y. P.; Stöckigt, J. *Chem. Asian J.* **2010**, *5*, 2400−2404.

(4) (a) Ma, X. Y.; Koepke, J.; Fritzsch, G.; Diem, R.; Kutchan, T. M.; Michel, H.; Stöckigt, J. *Biochim. Biophys. Acta* **2004**, *1702*, 121−124. (b) Maresh, J. J.; Giddings, L. A.; Friedrich, A.; Loris, E. A.; Panjikar, S.; Trout, B. L.; Stöckigt, J.; Peters, B.; O'Connor, S. E. *J. Am. Chem. Soc.* **2008**, *130*, 710−723.

(5) Stöckigt, J.; Antonchick, A. P.; Wu, F. R.; Waldmann, H. *Angew. Chem., Int. Ed.* **2011**, *50*, 8538−8564.

(6) (a) Stöckigt, J.; Zenk, M. H. *J. Chem. Soc., Chem. Commun.* **1977**, 646−648. (b) Nagakura, N.; Rüffer, M.; Zenk, M. H. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2308−2312.

(7) Manske, R. H. F.; Holmes, H. L.; Rodrigo, R. G. A. *The Alkaloids, Chemistry and Physiology*; Academic Press: New York, 1981.

(8) (a) Kutchan, T. M.; Hampp, N.; Lottspeich, F.; Beyreuther, K.; Zenk, M. H. *FEBS Lett.* **1988**, *237*, 40−44. (b) Kutchan, T. M.; Dittrich, H.; Bracher, D.; Zenk, M. H. *Tetrahedron* **1991**, *47*, 5945−5954. (c) Kutchan, T. M. *Phytochemistry* **1993**, *32*, 493−506.

(9) (a) Chang-Fong, J.; Tyacke, R. J.; Lau, A.; Westaway, J.; Hudson, A. L.; Glennon, R. A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1003−1005. (b) Tiwari, R. K.; Verma, A. K.; Chhillar, A. K.; Singh, D.; Singh, J.; Sankar, V. K.; Yadav, V.; Sharma, G. L.; Chandra, R. *Bioorg. Med. Chem.* **2006**, *14*, 2747−2752. (c) Goldberg, D. R.; et al. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 938−941.

(10) (a) Abbiati, G.; Arcadi, A.; Bellinazzi, A.; Beccalli, E.; Rossi, E.; Zanzola, S. *J. Org. Chem.* **2005**, *70*, 4088−4095. (b) Bandini, M.; Eichholzer, A.; Tragni, M.; Umani-Ronchi, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 3238−3241. (c) Laliberte, S.; Dornan, P. K.; Chen, A. *Tetrahedron Lett.* **2010**, *51*, 363−366.

(11) (a) Fridrichsons, J.; Mathieson, A. M. *Acta Crystallogr.* **1967**, *23*, 439−448. (b) Garg, N. K.; Sarpong, R.; Stoltz, B. M. *J. Am. Chem. Soc.* **2002**, *124*, 13179−13184. (c) Garg, N. K.; Stoltz, B. M. *Chem. Commun.* **2006**, *36*, 3769−3779.

(12) Battersb, A. R.; Burnett, A. R.; Parsons, P. G. *J. Chem. Soc.* **1969**, *8*, 1193−1200.

(13) Patthy-Lukats, A.; Kocsis, A.; Szabo, L. F.; Podanyi, B. *J. Nat. Prod.* **1999**, *62*, 1492−1499.

(14) Li, J. W.-H.; Vederas, J. C. *Science* **2009**, 161−165.