Characterization of Terfestatin A, a New Specific Inhibitor for Auxin Signaling.

Atsushi Yamazoe, Ken-ichiro Hayashi, Stefan Kepinski, Ottoline Leyser, and Hiroshi Nozaki*

From the Department of Biochemistry, Okayama University of Science, 1-1 Ridai-cho, Okayama City 700-0005 Japan (A.Y., K-I.H., H.N.); and Department of Biology, University of York, Box 373, York YO10 5YW, United Kingdom (S.K., O.L.)

Running title: A new specific inhibitor of auxin action

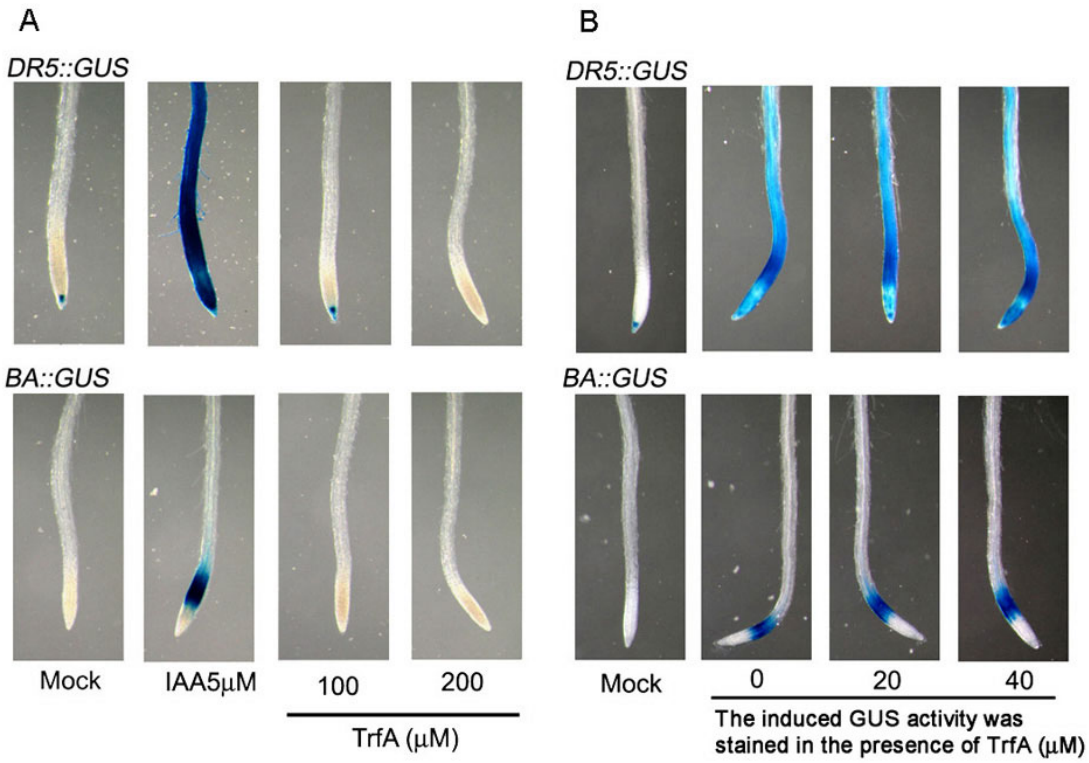
‡ To whom correspondence should be addressed: Department of Biochemistry, Okayama University of Science, 1-1 Ridai-cho, Okayama City 700-0005 Japan. Tel.: +81-86-256-9661; Fax: +81-86-256-9559; E-mail: hayashi@dbc.ous.ac.jp

CONTENTS:

1. Supplemental Figures 1 - 6.

Supplemental Figure 1.

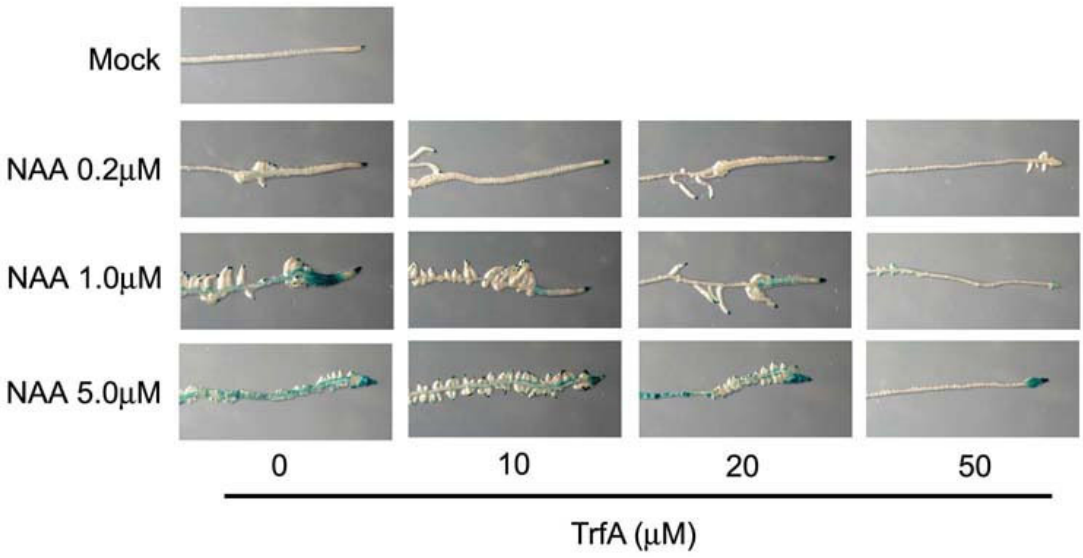
Terfestatin A (TrfA) had no effects on the induction of auxin-responsive gene expression and the GUS enzymatic activity.



A, Five-day-old *BA::GUS* or *DR5::GUS* seedlings were treated with 5μM IAA or with TrfA for 12 h. After the induction, the seedlings were washed with a GUS-staining buffer and transferred to the GUS-staining buffer containing 1 mM X-Gluc, the substrate for histochemical staining and incubated at 37 °C until sufficient staining developed.

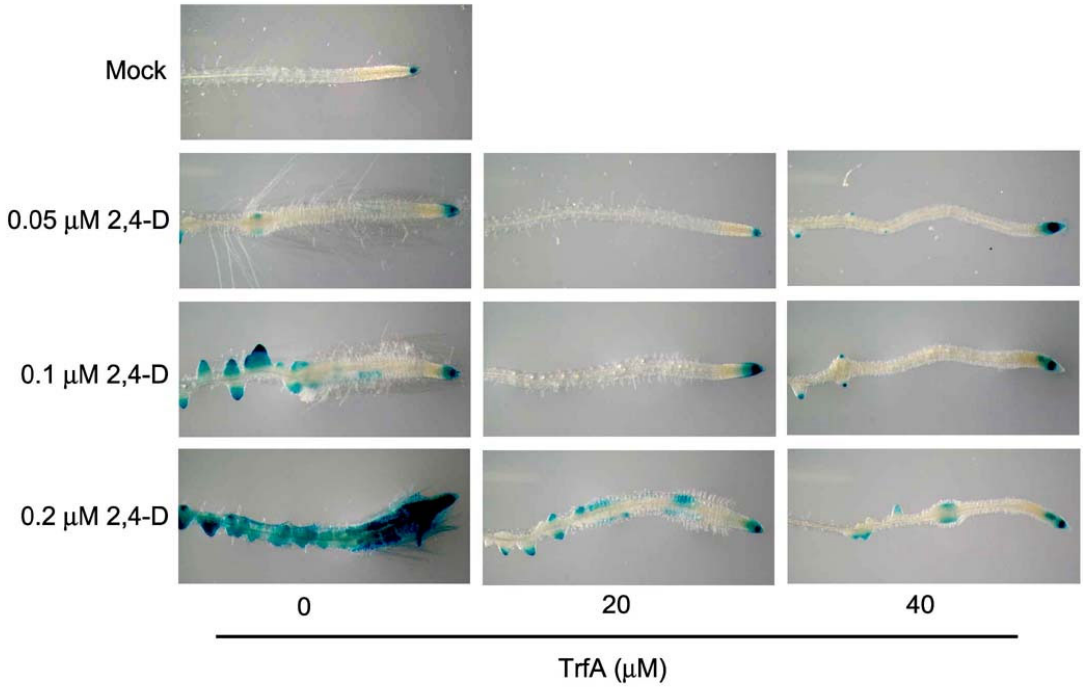
B, The IAA-induced GUS enzymatic activity was measured in the presence of TrfA to examine the effect of TrfA on GUS enzyme activity. 5-day-old *BA::GUS* or *DR5::GUS* seedlings were treated with 5μM IAA for 7 h. After the induction, the induced GUS activity was stained by the GUS-staining buffer containing 1 mM X-Gluc and 20 or 40 μM TrfA.

Supplemental Figure 2.
TrfA counteracted NAA on auxin-induced lateral root formation and *DR5::GUS* expression.



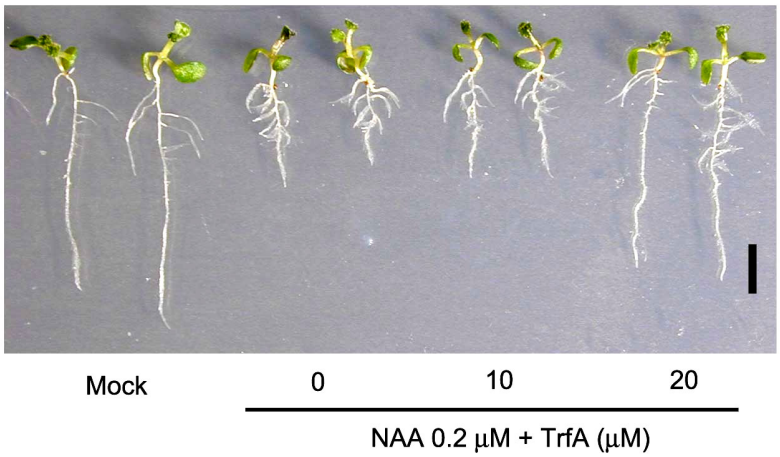
Four-day-old *DR5::GUS* seedlings were transferred to liquid GM media containing various concentrations of NAA and/or TrfA and incubated for additional 3 days. After cultivation, GUS expression was visualized by X-Gluc.

Supplemental Figure 3.
TrfA counteracted 2,4-D on auxin-induced lateral root formation and *DR5::GUS* expression.



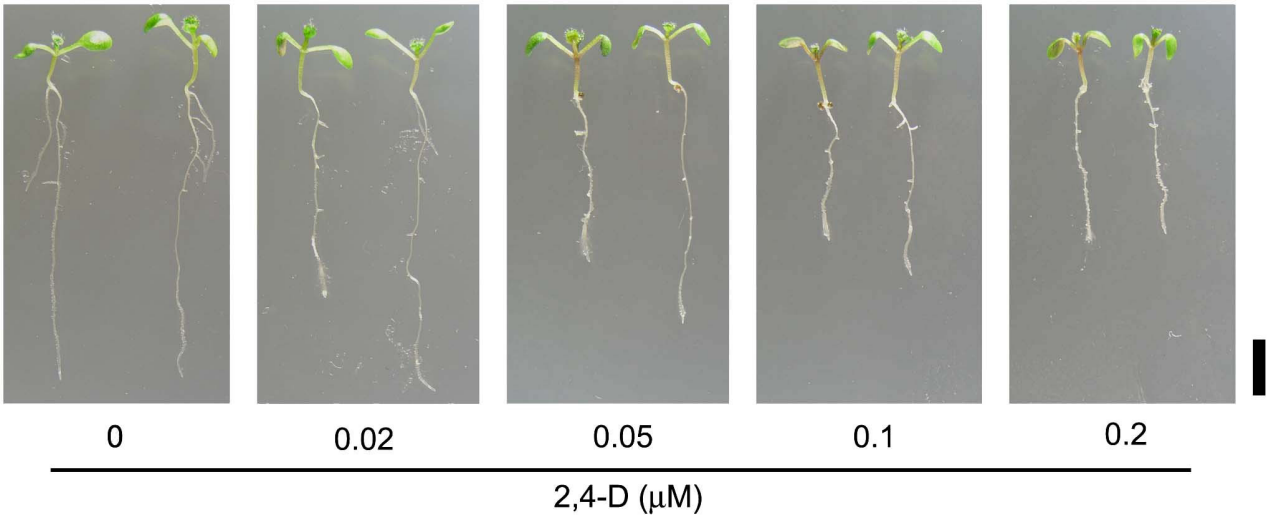
Four-day-old *DR5::GUS* seedlings were transferred to liquid GM media containing various concentrations of 2,4-D and/or TrfA and incubated for additional 3 days. After cultivation, GUS expression was visualized by X-Gluc.

Supplemental Figure 4.
TrfA restored NAA-induced primary root inhibition.



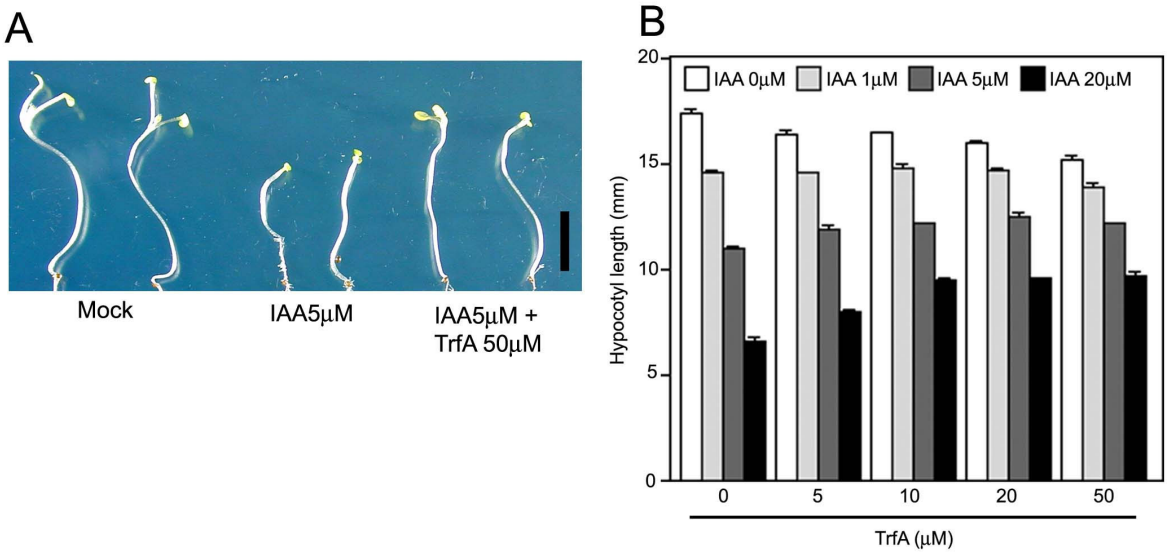
Three-day-old seedlings were transferred to liquid GM media containing various concentrations of TrfA in the presence of 0.2 μM NAA and incubated for additional 4 days. Bar represents 5 mm.

Supplemental Figure 5.
TrfA restored 2,4-D induced primary root inhibition.



Four-day-old seedlings were transferred to liquid GM media containing various concentrations of 2,4-D in the absence (left seedling) or presence (right seedling) of 20 μM TrfA and incubated for additional 3 days under continuous light. Bar represents 5 mm.

Supplemental Figure 6.
TrfA counteracted IAA on auxin-induced hypocotyl inhibition.



A. Three-day-old etiolated seedlings were transferred into liquid GM medium containing 50 μM TrfA in the presence of 5 μM IAA and then incubated for another 4 days in dark. Bar represents 5 mm.

B. Three-day-old seedlings were transferred into liquid GM medium containing the indicated concentration of TrfA with or without various concentrations of IAA. The hypocotyl length was measured after another 4 days culture in dark. Error bars, S.E.