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Brief Report

Synthesis and herbicidal activity of 3-substituted toxoflavin analogs

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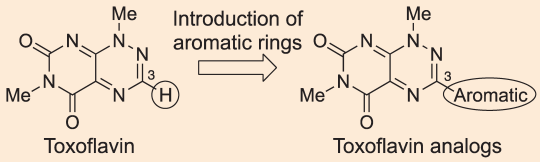
Supplementary material

We investigated the synthesis and herbicidal activity of 23 toxoflavin analogs, **1a**–**w**, in which aromatic rings (R) were introduced into the C-3 position. In paddy field conditions, **1k** (R=2-CF_3–C_6H_4) and **1w** (R=2-thienyl) showed excellent herbicidal activity. Under upland field conditions, we found that toxoflavin analogs **1a** (R=C_6H_5), **1n** (R=2-CH_3O–C_6H_4), and **1p** (R=4-CH_3O–C_6H_4) exhibited wide herbicidal spectrum against *Echinochloa crus-galli (L) var. crus-galli* (ECHCG), *Chenopodium album*, and *Amaranthus viridis* (AMAVI). The analog with the 2-fluoro group on benzene ring **1b** also showed high herbicidal activity against both ECHCG and AMAVI.



Keywords: Toxoflavin analogs, herbicidal activity.

Introduction

Toxoflavin was first reported by van Veen and Mertens in 1934 as a toxin produced by *Pseudomonas cocovenenans* (Fig. 1).[1)] This compound is responsible for the pathogenicity of bacteria, such as *Burkholderia glumae*, that cause diseases in various plants, including rice.[2,3)] Moreover, reumycin and fervenulin have also been isolated from *B. glumae* (Fig. 1).[3)] Toxoflavin is an extremely strong electron transmitter that bypasses the electron transport chain and produces hydrogen peroxide in the presence of oxygen.[1)] Therefore, it is highly toxic to organisms apart from yeast.[4)] Additionally, bromacil, terbacil, lenacil, saflufenacil, tiafenacil, metribuzin, and metamitron, which are currently used as herbicides, contain uracil and 1,2,4-triazine. The former five and the latter two herbicides have been reported as inhibitors of photosynthesis at PS II (HRAC Code: 5) and protoporphyrinogen oxidase (HRAC Code: 14), respectively.[5–9)] Therefore, toxoflavin is a potential herbicide, as the skeleton of toxoflavin is constructed by the fusion of uracil and 1,2,4-triazine rings. Although the synthesis of toxoflavin and its analogs has been reported,[10–16)] the structure–activity relationship (SAR) for their herbicidal activity is uncertain. Here, we found several herbicidally active toxoflavin analogs and identified their SARs. Synthetic analogs are also expected to exhibit the inhibitory activity described above.

Materials and methods

1. *Synthesis of toxoflavin analog **1j** (general procedure)*

*1.1. 3-Methyl-6-(1-methyl-2-(4-methylbenzylidene)-hydrazineyl)pyrimidine-2,4(1*H*,3*H*)-dione* (***3j***)

To a solution of hydrazinouracil (**2**, 613 mg, 3.53 mmol) in ethanol (10 mL), *p*-tolualdehyde (0.46 mL, 3.9 mmol) was added. After being stirred at reflux for 2 hr, the resulting precipitate was filtered to give imine (**3j**, 793 mg, 83%).

1.2. 1,6-Dimethyl-3-(p-*tolyl)pyrimido*[*5,4*-e][*1,2,4*]*triazine-5,7(1*H*,6*H*)-dione* (***1j***)

A solution of imine (**3j**, 308 mg, 1.10 mmol) in acetic acid (4 mL) was added to an ice-cold solution of sodium nitrite (126 mg, 1.65 mmol) in water (0.22 mL). After stirring at room temperature for 3 hr, ether was added to the reaction mixture. The resulting orange crystals were filtered to give a mixture of toxoflavin analog **1j** and *N*-oxide **4j**, which was used for the next reaction without further purification.

To a solution of **1j** and **4j** in ethanol (3 mL), dithiothreitol (253 mg, 1.64 mmol) was added. After being stirred for 14 hr at room temperature, the reaction mixture was added to ether. The resulting precipitate was filtered to give the 140 mg (93%) of the

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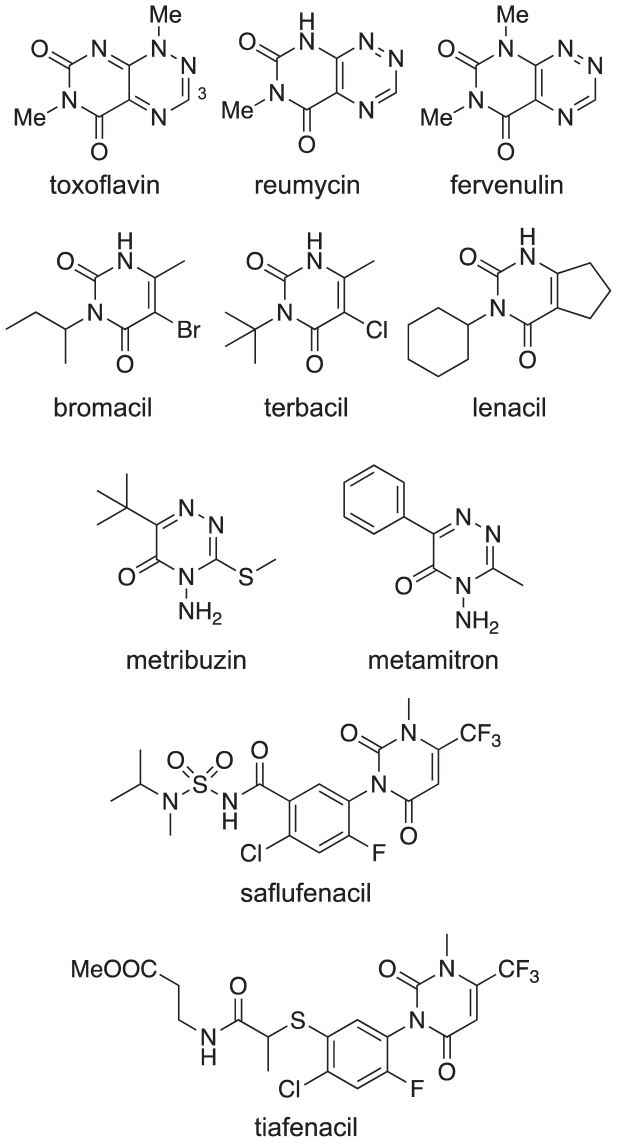


Fig. 1. Products isolated from *Burkholderia glumae* and current herbicides having uracil and 1,2,4-triazine

toxoflavin analog (**1j**).

1j: mp 227–229°C; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 2.40 (s, 3H), 3.28 (s, 3H), 4.04 (s, 3H), 7.40 (d, J=7.5 Hz, 2H), 8.09 (d, J=7.5 Hz, 2H). Other analogs were synthesized using the same method as described above. For other data of toxoflavin analogs, see Supplementary data S1.

2. Evaluation of herbicidal activity on weeds of rice paddy fields

Plastic pots (100 cm^2) were filled with paddy soil (clay loam). Water, fertilizer, and soil puddling were added successively. Seeds of *Echinochloa crus-galli (L) var. formosensis* (ECHCS), *Monochoria vaginalis* (MOOVA), and *Lindernia procumbens* (LIDPY) were sown on the soil surface. The pots were filled within 3 cm of the rim with water. Each test compound was dissolved in a mixture of acetone, polyoxyethylene styryl phenyl ether, and calcium dodecylbenzene sulfonate to give an emulsifiable concentrate. An amount of the water-diluted agent solutions was sprayed on the water surface 7 days after sowing for application. The dosage of the compounds was 1200 g/ha. These test plants were grown in a greenhouse for 14 days. Afterward, the herbicidal activity was visually evaluated using the following scale: 0 (no effect) to 100 (completely effective).

3. Evaluation of herbicidal activity on weeds of upland fields

Plastic pots (36 cm^2) were filled with dry field farming soil (loam, clay). Seeds of *Echinochloa crus-galli (L) var. crus-galli* (ECHCG), *Chenopodium album* (CHEAL), and *Amaranthus viridis* (AMAVI) were sown at a depth of 1 cm. Each test compound was prepared according to the evaluation of weeds in rice paddy fields. The water-diluted agent solutions were sprayed onto plants 7 days after sowing for post-emergence application. The spray volume was equivalent to 1000 L/ha, and the compound dosage was 1200 g/ha. The test plants were grown and their herbicidal activity evaluated in the same way as the weeds of rice paddy fields.

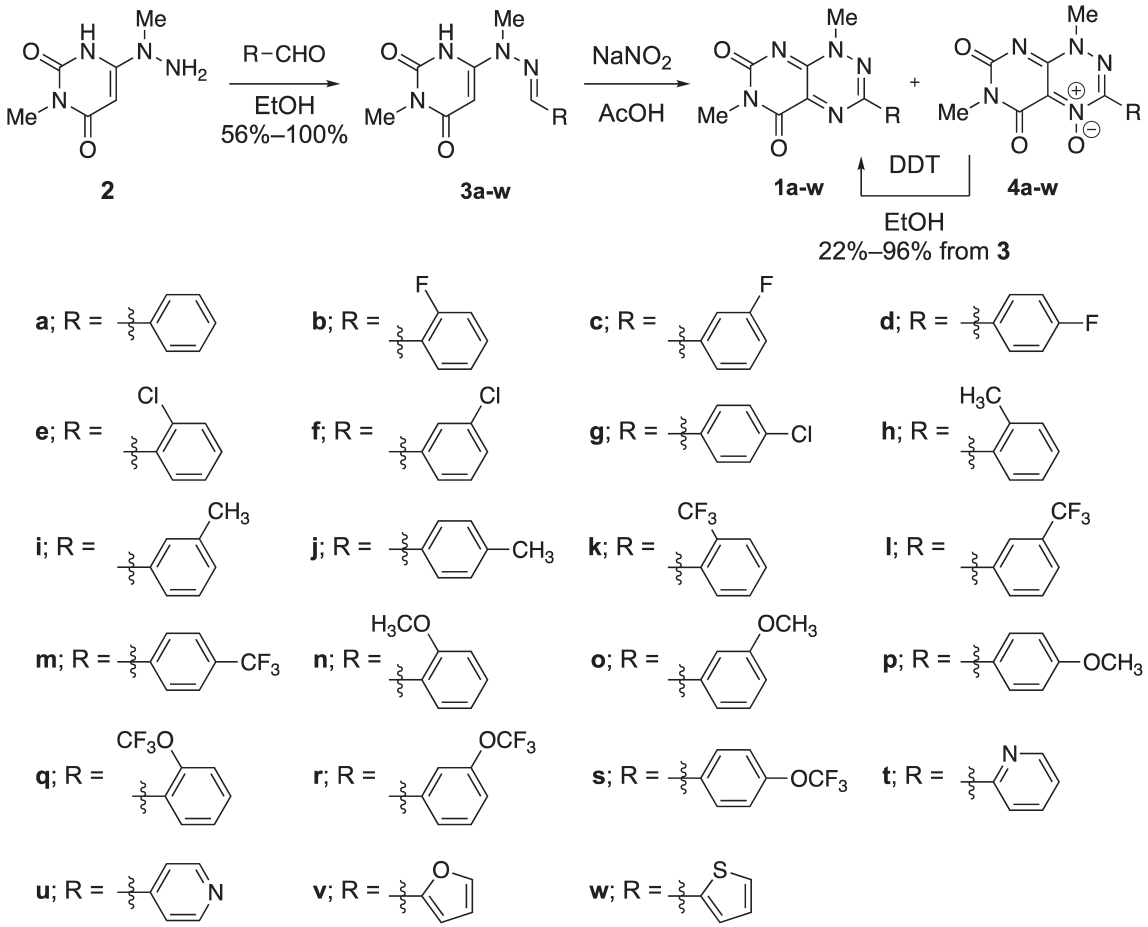
Results and discussion

*1. Synthesis of 3-substituted toxoflavin analogs **1a–w***

The molecular design of the toxoflavin analogs can be considered at the following two points: (1) the conversion of the two methyl groups to different substituents, or (2) the introduction of a substituent at the C-3 position. In particular, a variety of substituents can be easily introduced at the C-3 position. Therefore, we decided to introduce a substituent at the C-3 position as the first target molecule. In this paper, we focused on the effect of the type of aromatic substituent on herbicidal activity. Here, the method for synthesizing toxoflavin analogs reported by Hollis Showalter *et al.* and Mao *et al.*[14,16] was modified. The imination of hydrazinouracil **2** with various aldehydes gave the corresponding hydrazones (**3a–w**, Scheme 1 and Supplemental Scheme S1). At this time, the geometry of the carbon–nitrogen double bond of **3a–w** was not determined. Subsequently, hydrazones **3a–w** were reacted with sodium nitrite to afford 3-substituted toxoflavin analogs **1a–w**. However, along with these analogs, *N*-oxides **4a–w** were also obtained. The ratios of **1a–w** and **4a–w** were 1 : 1 to 1 : 5, as determined by ^{1}H NMR spectrum. Each mixture of **1a–w** and **4a–w** was reduced with dithiothreitol to obtain the desired toxoflavin analogs **1a–w** as the sole product.

2. Herbicidal activity of toxoflavin analogs

The herbicidal activity of the synthesized toxoflavin analogs **1a–w** was evaluated on three species of weeds in rice paddy field conditions (ECHCS: *Echinochloa crus-galli (L) var. formosensis*; MOOVA: *Monochoria vaginalis*; and LIDPY: *Lindernia procumbens*) and upland field conditions (ECHCG: *Echinochloa crus-galli (L) var. crus-galli*; CHEAL: *Chenopodium album*; and AMAVI: *Amaranthus viridis*) (Table 1). All synthesized compounds were applied by spraying methods: water surface spraying for paddy weeds and leaf or soil spraying for upland weeds.

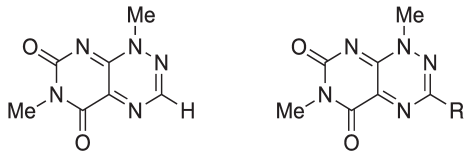


Scheme 1. Synthesis of toxoflavin analogs (**1a**–**w**)

First, we examined the herbicidal activity against the three paddy weeds (ECHCS, MOOVA, and LIDPY) in a pot trial. Toxoflavin showed slight herbicidal activity against ECHCS but was completely ineffective against other weeds tested. Compound **1a**, having a phenyl group at the C-3 position of toxoflavin, controlled both MOOVA and LIDPY growth completely, but the herbicidal activity of **1a** for ECHCS was low. Compound **1d** showed moderate activity against ECHCS and LIDPY, but other halogenated analogs—**1b**, **1c**, and **1e**–**g**—were not effective for paddy weeds. Toxoflavin analogs **1h**–**j** with a tolyl group maintained moderate activity for ECHCS, and 3-methyl analog **1i** showed a broad herbicidal spectrum. Among compounds with a hydrophobic substituent, such as a trifluoromethyl group **1k**–**m**, the 2-CF_3 analog **1k** gave good results in our evaluation for paddy field conditions; thus, it was moderately active against ECHCS and killed MOOVA and LIDPY completely with an application of 1200 g/ha. Moreover, it was found that the introduction of a CF_3 group at the *ortho*-position on the benzene ring provided increased activity against ECHCS as compared to the herbicidal activity of compound **1a**. Conversely, toxoflavin analogs **1n**–**s** with a methoxy group and trifluoromethoxy group showed no activity. To improve water solubility, we introduced pyridine rings **1t** and **1u** into toxoflavin with no improvement in activity. Next, we synthesized analogs **1v** and **1w** introduced with 2-furanyl and 2-thienyl, known as 5-membered aromatic compounds, for comparison with **1a**. Thienyl analog **1w** showed moderate activity against ECHCS and killed MOOVA and LIDPY completely.

Next, we examined the herbicidal activity of toxoflavin analogs against three species of weeds (ECHCG, CHEAL, AMAVI) under upland field conditions. Toxoflavin showed potent herbicidal activity for ECHCG in our upland evaluation and had moderate activity against CHEAL and AMAVI. As seen in the case of **1a**, the introduction of a phenyl ring at the C-3 position of toxoflavin improved herbicidal activity against AMAVI, and we were able to control their growth well. All fluorinated analogs **1b**–**d** provided high levels of herbicidal activity for AMAVI, and the 2-fluorinated analog **1b** was also active against ECHCG. Chlorophenyl analogs **1e**–**g** did not yield better results than those of fluorophenyl compounds **1b**–**d**. Although toxoflavin analogs with 3-tolyl group **1i** had a broad spectrum in the paddy field condition test, all tolyl analogs **1h**–**j** showed moderate to high levels of activity against all weeds in our upland condition without regard for the position of a methyl group on the benzene ring. Results for the substitution of a trifluoromethyl group were not good, except for the ECHCG-controlling activ-

Table 1. Herbicidal activity of toxoflavin analogs (**1a–w**)



Toxoflavin **1a-w**

No.		Herbicidal activity [a–c]					
		Paddy/water surface application			Upland/foliage or soil application		
		Early-Post emergence					
	R	ECHCS	MOOVA	LIDPY	ECHCG	CHEAL	AMAVI
Toxoflavin	H	40	0	0	90	60	40
1a	C_6H_5	0	100	100	100	40	100
1b	2-F-C_6H_4	0	0	0	100	0	100
1c	3-F-C_6H_4	0	0	0	0	0	100
1d	4-F-C_6H_4	40	0	60	50	40	100
1e	2-Cl-C_6H_4	0	0	0	100	40	50
1f	3-Cl-C_6H_4	0	0	0	0	0	0
1g	4-Cl-C_6H_4	0	0	0	60	70	70
1h	2-CH_3-C_6H_4	40	0	0	80	90	70
1i	3-CH_3-C_6H_4	60	60	70	50	40	80
1j	4-CH_3-C_6H_4	50	0	0	70	60	90
1k	2-CF_3-C_6H_4	50	100	100	100	0	0
1l	3-CF_3-C_6H_4	40	40	0	0	0	40
1m	4-CF_3-C_6H_4	0	0	0	0	0	0
1n	2-CH_3O-C_6H_4	0	0	0	70	90	90
1o	3-CH_3O-C_6H_4	0	0	0	0	0	0
1p	4-CH_3O-C_6H_4	0	0	0	80	40	90
1q	2-CF_3O-C_6H_4	0	0	0	0	0	0
1r	3-CF_3O-C_6H_4	0	0	0	0	0	0
1s	4-CF_3O-C_6H_4	0	0	0	0	0	100
1t	2-Pyridyl	0	0	0	70	70	60
1u	4-Pyridyl	0	0	0	60	60	80
1v	2-Furanyl	0	0	0	0	0	0
1w	2-Thienyl	40	100	100	40	0	80

[a] Rating scale: 0 (no effect)–100 (completely effective). [b] Dose of sample: 1200 g a.i./hectare. [c] ECHCS (*Echinochloa crus-galli (L) var.formosensis*), MOOVA (*Monochoria vaginalis*), LIDPY (*Lindernia procumbens*) ECHCG (Echinochloa *crus-galli (L) var.crus-galli*), CHEAL (*Chenopodium album*), AMAVI (*Amaranthus viridis*)

ity of compound **1k**. The 2- or 4-methoxy analogs **1n** and **1p** were moderately to highly active for all upland weeds tested; however, the 3-methoxy analog was inactive. Substitution of a trifluoromethyl group tended to lower the activity. Both pyridyl compounds **1t** and **1u** showed moderate activity; in contrast, the 2-furanyl compound was inactive. We found that toxoflavin and its analogs had higher herbicidal activity in uplands than in paddy fields. In this study, we considered that anaerobic conditions in paddy fields and aerobic conditions in upland fields could account for the difference, since toxoflavin is reported to exhibit biological activity under aerobic conditions.[1)]

Conclusion

Toxoflavin analogs with an aromatic ring at the C-3 position were synthesized and subjected to weeding tests in rice paddy and upland field conditions. We have revealed that: (1) the introduction of a benzene ring onto the C-3 position of toxoflavin improved its herbicidal activity, and (2) the introduction of single substituent on the benzene ring of **1a** or the replacement of the benzene ring of **1a** with a pyridyl or a thienyl group broadened the herbicidal spectrum of **1a**. We believe that these results are significant for the development of new herbicides for use in rice paddy and upland fields. Future research will be conducted to continue identification of the active site of toxoflavin and de-

velop toxoflavin analogs with stronger herbicidal activity.

Electronic supplementary materials

The online version of this article contains supplementary material (EMS1-3), which is available at https://www.jstage.jst.go.jp/browse/jpestics/.

References

1) H. E. Latuasan and W. Berends: On the origin of toxicity of toxoflavin. *Biochim. Biophys. Acta* **52**, 502–508 (1961).
2) R. A. Machlowitz, W. P. Fisher, B. S. McKay, A. A. Tytell and J. Charney: Xanthothricin, a new antibiotic. *Antibiot. Chemother. (Northfield Ill.)* **4**, 259–261 (1954).
3) Z. Sato, Y. Koiso, S. Iwasaki, I. Matsuda and A. Shirata: Toxins produced by *Pseudomonas glumae*. *Ann. Phytopathol. Soc. Jpn.* **55**, 353–356 (1989) (in Japanese) https://doi.org/10.3186/jjphytopath.55.353.
4) K. Iiyama, N. Furuya, Y. Takanami and N. Matsuyama: A role of phytotoxin in virulence of *Pseudomonas glumae* Kurita et Tabei. *Ann. Phytopathol. Soc. Jpn.* **61**, 470–476 (1995) https://doi.org/10.3186/jjphytopath.61.470.
5) D. Yanase and A. Andoh: Translocation of photosynthesis-inhibiting herbicides in wheat leaves measured by phytofluorography, the chlorophyll fluorescence imaging. *Pestic. Biochem. Physiol.* **44**, 60–67 (1992).
6) J. Park, Y. O. Ahn, J.-W. Nam, M.-K. Hong, N. Song, T. Kim, G.-H. Yu and S.-K. Sung: Biochemical and physiological mode of action of tiafenacil, a new protoporphyrinogen IX oxidase-inhibiting herbicide. *Pestic. Biochem. Physiol.* **152**, 38–44 (2018).
7) K. Grossmann, R. Niggeweg, N. Christiansen, R. Looser and T. Ehrhardt: The herbicide saflufenacil (KixorTM) is a new inhibitor of protoporphyrinogen IX oxidase activity. *Weed Sci.* **58**, 1–9 (2010).
8) J. H. Eley, J. F. McConnell and R. H. Catlett: Inhibition of metribuzin on growth and photosynthesis of the blue-green alga *Anacystis nidulans*. *Environ. Exp. Bot.* **23**, 365–368 (1983).
9) R. A. Stern: The photosynthesis inhibitor metamitron is an effective fruitlet thinner for 'Gala' apple in the warm climate of Israel. *Sci. Hortic. (Amsterdam)* **178**, 163–167 (2014).
10) G. D. Daves Jr., R. K. Robins and C. C. Cheng: The total synthesis of toxoflavin. *J. Am. Chem. Soc.* **83**, 3904–3905 (1961).
11) F. Yoneda and T. Nagamatsu: A convenient synthesis of toxoflavins, toxoflavin 4-oxides and 1-demethyltoxoflavins. *Chem. Pharm. Bull.* **23**, 2001–2009 (1975).
12) A. J. Turbiak and H. Showalter: A novel synthesis of N_1-(substituted)-pyrimido[5,4-*e*]-1,2,4-triazine-5,7(1*H*,6*H*)-diones. *Tetrahedron Lett.* **50**, 1996–1997 (2009).
13) N. Todorovic, A. Giacomelli, J. A. Hassell, C. S. Frampton and A. Capretta: Microwave-assisted synthesis of 3-aryl-pyrimido[5,4-*e*][1,2,4]triazine-5,7-(1*H*,6*H*)-dione libraries: Derivatives of toxoflavin. *Tetrahedron Lett.* **51**, 6037–6040 (2010).
14) J. Zeller, A. J. Turbiak, I. A. Powelson, S. Lee, D. Sun, H. D. H. Showalter and E. R. Fearon: Investigation of 3-aryl-pyrimido[5,4-*e*][1,2,4]triazine-5,7-diones as small molecule antagonists of β-catenin/TCF transcription. *Bioorg. Med. Chem. Lett.* **23**, 5814–5820 (2013).
15) A. Raoof, P. Depledge, N. M. Hamilton, N. S. Hamilton, J. R. Hitchin, G. V. Hopkins, A. M. Jordan, L. A. Maguire, A. E. McGonagle, D. P. Mould, M. Rushbrooke, H. F. Small, K. M. Smith, G. J. Thomson, F. Turlais, I. D. Waddell, B. Waszkowycz, A. J. Watson and D. J. Ogilvie: Toxoflavins and deazaflavins as the first reported selective small molecule inhibitors of tyroxyl-DNA phosphodiesterase II. *J. Med. Chem.* **56**, 6352–6370 (2013).
16) Y. Mao, W. Tian, Z. Huang and J. An: Convenient synthesis of toxoflavin that targets β-catenin/Tcf4 signaling activities. *J. Heterocycl. Chem.* **51**, 594–596 (2014).