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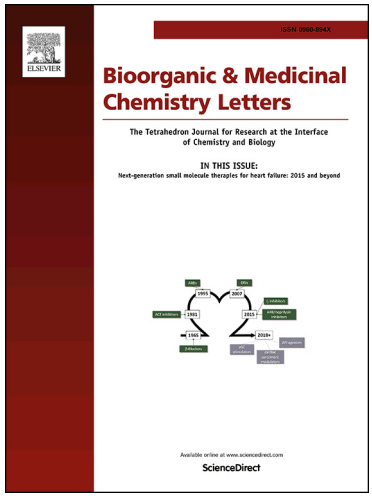

3*H*-Imidazo[4,5-b]pyridine-6-carboxylic Acid Derivatives as Rexinoids with Reduced Teratogenicity

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3*H*-Imidazo[4,5-b]pyridine-6-carboxylic Acid Derivatives as Rexinoids with Reduced Teratogenicity

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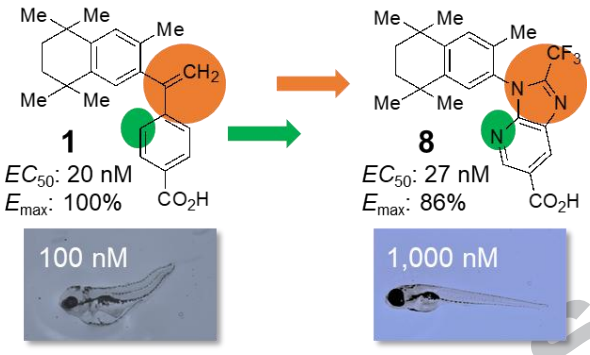
ABSTRACT: Several retinoid X receptor (RXR) ligands (rexinoids), such as bexarotene (**1**), exhibit teratogenicity, which is a serious impediment to their clinical application. We considered that rexinoids with a lower level of maximal RXR transcription activation (i.e., partial agonists) and lower lipid solubility might show weaker adverse side effects. Based on this idea, we modified our previously reported pentamethyltetralin-type RXR partial agonists **5** and **6** to reduce their lipophilicity. Here, we report a new RXR partial agonist, 3-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-2-(trifluoromethyl)-3*H*-imidazo[4,5-b] pyridine-6-carboxylic acid (**8**, CATF-PMN), which showed greatly reduced teratogenicity in zebrafish embryos.

KEYWORDS: Nuclear receptor, RXR, rexinoid, teratogenicity, zebrafish

Table of Contents Graphic



The incidence of birth defects in neonates is about 2–3%,[1] and it is reported that about 1–3% of them are caused by medicines.[2] A well-known example of teratogenicity as a drug side effect is provided by thalidomide, which was marketed in 1957 as a remedy for morning sickness in pregnant women and as a sleeping drug for infants, but was subsequently found to cause birth defects (thalidomide embryopathy).[3,4] Various vitamin A-related compounds (retinoids) also show teratogenicity via activation of retinoic acid receptors (RARs),[5-8] especially RARα.[9] Thus, assessment of teratogenic potential is an essential part of drug development.

Zebrafish (*Danio rerio*) are used as a model for prediction of the teratogenic potential of compounds in humans.[10] Zebrafish are useful models for studying various bioprocesses of vertebrates because of their genetic proximity to humans and the transparency of their eggs and larvae.[11] For example, bexarotene (**1**, Figure 1), used to treat cutaneous T cell lymphoma (CTCL),[12] was found to be teratogenic in zebrafish.[13] The molecular targets of **1** are retinoid X receptors (RXRs), and other RXR activators such as 9-*cis* retinoic acid (**2**) and triphenyltin (TPT, **3**, Figure 1) also exhibit teratogenicity.[13] It is reported that **2** can activate not only RXR but also RAR.[14] For example, **1** is an RXR agonist that also activates RAR.[15] This dual activity results in a synergistic effect,[16] which may account for the teratogenicity of these compounds. Interestingly, the RXR

antagonist UVI3003 (**4**, Figure 1) is also teratogenic.[17] Therefore, RXR activation itself is unlikely to be involved in teratogenicity. In fact, the teratogenicity of **4** was reported to be caused by PPARγ (peroxisome proliferator-activated receptor γ) activation.[17] On the other hand, pioglitazone hydrochloride, a well-known PPARγ agonist, is not teratogenic in zebrafish.[18] Since pioglitazone is used as pioglitazone hydrochloride, we speculated that its high molecular polarity might account for the lack of teratogenicity. Actually, it is reported that the solubilities of pioglitazone and pioglitazone hydrochloride in water are 0.039 mM and 0.70 mM, respectively.[19] Therefore, we hypothesized that RXR agonists with reduced teratogenicity might be obtained by increasing their molecular polarity or hydrophilicity. Here, we report the successful application of this idea to obtain novel RXR ligands with reduced teratogenicity.

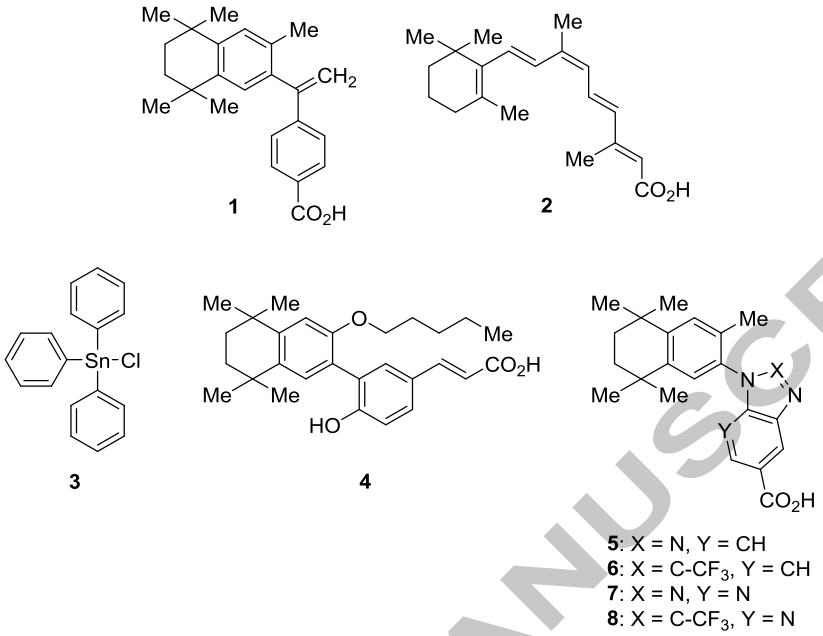


Figure 1. Chemical structures of **1**–**8**.

Based on the fact that highly lipophilic full RXR agonists, such as **1**, show teratogenicity, we first examined the teratogenicity of our previously reported RXR partial agonists CBt-PMN[20] (**5**, Figure 1) and CBTF-PMN[21] (**6**, Figure 1) in zebrafish. We focused on **5** and **6** because they show a lower blood triglyceride-elevating effect than bexarotene, so we considered that they might also show lower teratogenicity. These compounds have a similar pentamethyltetralin structure to **1**, but have lower lipophilicity (Table 1).

Table 1 shows the hatching rate, survival rate, and malformation rate in zebrafish after

exposure of the eggs to **1**, **5** or **6** for 120 hours. The group treated with **1** at 100 nM showed 67% hatching rate versus the untreated control, and the malformation rate was 100%. Malformations included spine curvature and an increased proportion of the head in the whole body (Figure 2). The groups treated with **5** or **6** at the same concentration showed hatching rates of 75% and 83%, respectively, but notably the malformation rate was 0% for both compounds. However, when the eggs were exposed to a higher concentration (1,000 nM) of **5** or **6**, the malformation rate increased to 100% and 17%, respectively. As regards RXR transcriptional activation, **6** shows a higher E_{max} value than **5** and the EC_{50} value of **6** is much larger than that of **5**. To examine whether the triazole structure of **5** might be associated with the teratogenicity and to examine the influence of increased molecular polarity, we therefore synthesized **7** and **8** (Figure 1) and evaluated their teratogenicity.

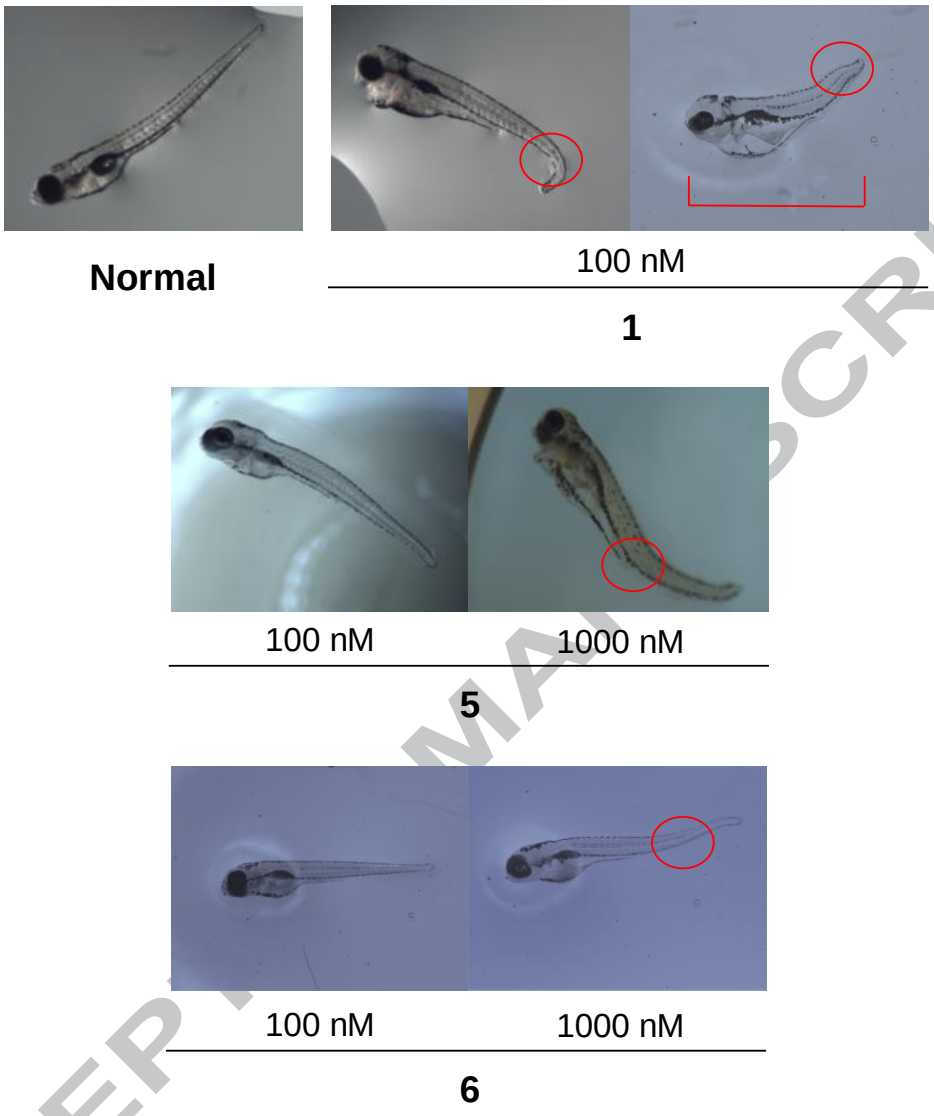


Figure 2. Representative photos of juvenile zebrafish exposed to RXR full agonist **1**, and partial agonists **5** and **6**. Compound **1** at 100 nM induced malformations such as spine curvature and head enlargement. Compounds **5** and **6** showed no effect at 100 nM, but at 1,000 nM they induced similar, though less marked, changes.

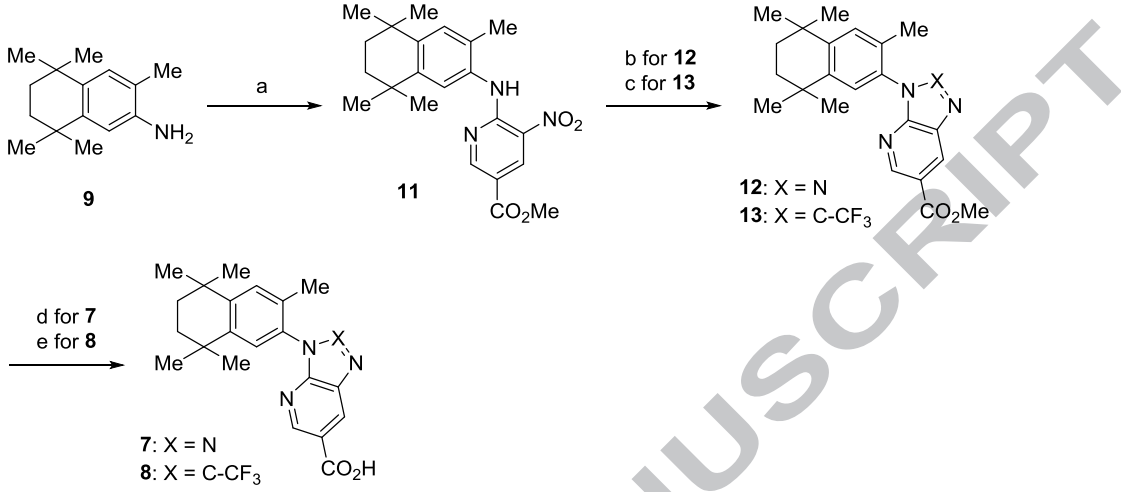
Table 1. Teratogenicity of RXR full agonist 1 and partial agonists 5 and 6 in zebrafish. Exposure to 5 and 6 was associated with higher hatching rates and lower malformation rates, compared with 1.

					RXR transcriptional activity		
	Conc. [nM]	Hatching [%]	Survival [%]	Teratogenicity [%]	EC_{50} [nM]	E_{max} [%]	Solubility [μM]
Normal	–	100	100	–	–	–	–
1	100	67	100	100	20	100	0.25
ref 12	287	99	99				
	100	75	100	0			
5	300	82	100	44	143	75	305
	1,000	100	100	100			
	100	83	100	0			
6	300	92	100	0	15	85	12.8
	1,000	100	100	17			

N = 9－12.

Scheme 1 illustrates the syntheses of **7** and **8**. The common precursor **9** for **5** and **6** was synthesized as reported previously (ref 16). Compound **9** was reacted with methyl 6-chloro-5-nitronicotinate (**10**) to afford **11**. After catalytic reduction of the nitro group of **11**, ring closure under appropriate conditions afforded **12**, which has a triazole skeleton, or **13**, which has an imidazole skeleton. Hydrolysis of the ester gave the target compounds **7** and **8**, respectively.

Scheme 1[a]



[a]Reagents and conditions: (a) methyl 6-chloro-5-nitronicotinate (**10**), MeOH, r.t., 4 h. (b) 1) H_2, Pd/C, EtOAc, r.t., 16 h. 2) $NaNO_2$, *dil.* H_2SO_4, THF, 5 °C, 1 h. (c) 1) H_2, Pd/C, EtOAc, r.t., 16 h. 2) trifluoroacetic anhydride, trifluoroacetic acid, r.t., 1 h. (d) 1) 2 N NaOH, MeOH, THF, 60 °C , 3 h. 2) 2 N HCl. (e) 1) 2 N NaOH, MeOH, 60 °C, 1.5 h. 2) 2 N HCl.

Table 2 summarizes the results of RXR transcriptional activation assay, water solubility measurement, and teratogenicity evaluation of **7** and **8.** Conversion of the benzoic acid derivatives **5** and **6** to nicotinic acid derivatives **7** and **8** increased the EC_{50} values, while the E_{max} values were little changed. The water solubility of **7** and **8** was significantly increased compared to that of **5** or **6**, respectively. Even when zebrafish embryos were exposed to the high concentration of 1,000 nM, the

malformation rate was 25% for **7** and 10% for **8**, respectively. At this concentration, individuals with marked spinal curvature were observed in the case of **7**, but not in the case of **8** (Figure 2). Since the results for **5** and **7** are rather similar, the teratogenicity may be related to the triazole skeleton. Furthermore, **6** and **8** showed similarly low rates of malformation at 1,000 nM. By analogy with pioglitazone hydrochloride, this suggests that high molecular polarity may reduce teratogenicity, possibly by decreasing the permeability of the compound across the zebrafish egg membrane, resulting in lower intra-egg exposure.

Table 2. Teratogenicity of nicotinic acid derivatives 7 and 8 in zebrafish. Compound 7 or 8 induced lower malformation rates than benzoic acid derivatives 5 and 6.

					RXR transcriptional activity		
	Conc. [nM]	Hatching [%]	Survival [%]	Teratogenicity [%]	EC_{50} [nM]	E_{max} [%]	Solubility [μM]
1	100	100	100	100	20	100	0.25
5	1,000	100	100	100	143	75	305
6	1,000	100	100	17	15	85	12.8
7	100	100	100	0	275	89	670
	300	100	100	0			
	1,000	100	100	25			
8	100	92	100	0	27	86	222
	300	100	100	0			
	1,000	83	100	10			

N = 9－12.

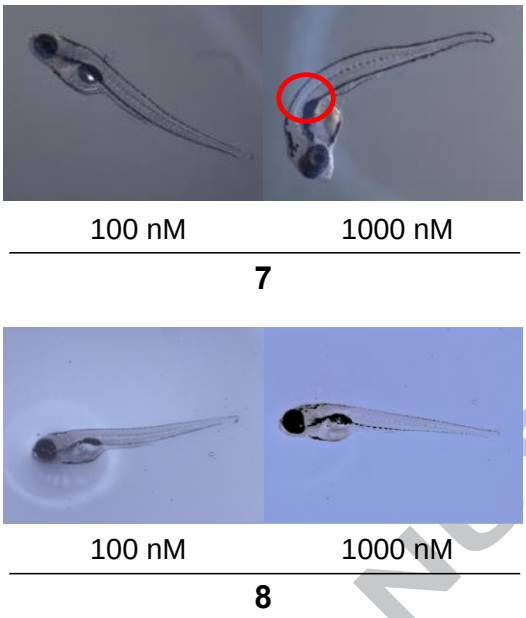


Figure 3. Representative photos of juvenile zebrafish exposed to nicotinic acid derivatives **7** and **8**. Compound **7** induced spinal curvature at the high concentration of 1,000 nM, but **8** did not induce any major malformation at this concentration.

In this study, we aimed to develop RXR agonists with reduced teratogenicity by modifying our previously reported RXR partial agonists CBt-PMN (**5**) and CBTF-PMN (**6**) to reduce their lipophilicity. We first established that **5** and **6** are less teratogenic than the full agonist bexarotene **1**, although **5** exhibited moderate teratogenicity. Conversion of the benzoic acid moiety of **5** and **6** to nicotinic acid to increase the molecular polarity and reduce the lipophilicity of these compounds

afforded **7** and **8**, which showed reduced teratogenicity, although **7** was still moderately teratogenic at high concentration. Compound **8** was identified as a new RXR partial agonist with greatly reduced teratogenicity, supporting the usefulness of our strategy of focusing on partial RXR agonists with reduced lipophilicity. More detailed teratogenicity evaluation using rodents or other models should be the next step to further validate this approach.

Associated contents

Supporting information

HPLC charts and NMR charts for compounds (PDF). The Supporting Information is available free of charge on the website.

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Author Contributions

H.K. conceived and designed the project. Y.T. and O.S. synthesized compounds. Y.T., M.T., M.N., M.W. performed zebrafish experiments. Y.T., M.W., and H.K. performed LRMS and HRMS. The manuscript was written by Y.T., M.W., and H.K. All authors analyzed and discussed the data.

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Notes

This research was partially performed in collaboration with AIBIOS Co., Ltd.. Michiko Fujihara is an employee of AIBOS. No other author reports any potential conflict of interest relevant to this article.

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Abbreviations

CTCL, cutaneous T cell lymphoma; EC_{50}, half-maximal effective concentration; E_{max}, efficacy maximal response; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor; RXR, retinoid X receptor; TPT, triphenyltin.

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