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Synthesis and structure–activity relationship of RXR antagonists based on the diazepinylbenzoic acid structure

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Abstract—Synthesis and structure–activity relationship of RXR antagonists employing a diazepinylbenzoic acid scaffold are described. Of those antagonists, sulfonamide derivatives (**6v** and **6w**) reveal a high antagonistic activity and good pharmacokinetic properties.

Retinoid X receptors (RXRs) are members of the nuclear receptor superfamily and act as ligand-inducible transcriptional factors.[1,2] RXRs regulate a wide variety of biological functions such as cell differentiation, proliferation, and embryonic development in vertebrates.[3] RXRs are divided into three sub-types α, β, γ and form a heterodimer with various other nuclear receptors (e.g., peroxisome proliferator-activated receptors: PPARs, liver X receptors: LXRs, farnesoid X receptor: FXR and retinoic acid receptors: RARs).[4]

HX531 (Fig. 1) was identified as an RXR antagonist on the basis of inhibitory activity on retinoid-induced cell differentiation of human promyelocytic cells HL-60 and transactivation assays using RARs and RXRs in COS-1 cells by Kagechika et al.[5] HX531 inhibited HL-60 cell differentiation induced by the combination of the retinoid agonist Am80 with an RXR agonist (a retinoid synergist, HX600), indicating that HX531 inhibited the activation of RAR–RXR heterodimers as well as homodimers. Kadowaki et al. reported that treatment of KKAy mice fed a high-fat diet with HX531 reduced plasma glucose and insulin levels, improved insulin sensitivity, and increased energy expenditure.

It also decreased body weight gain though no effect on food consumption was observed.[6] It suggested

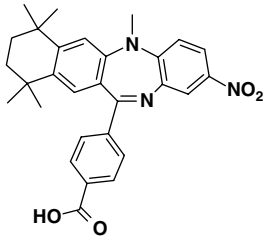

Figure 1. Structure of HX531.

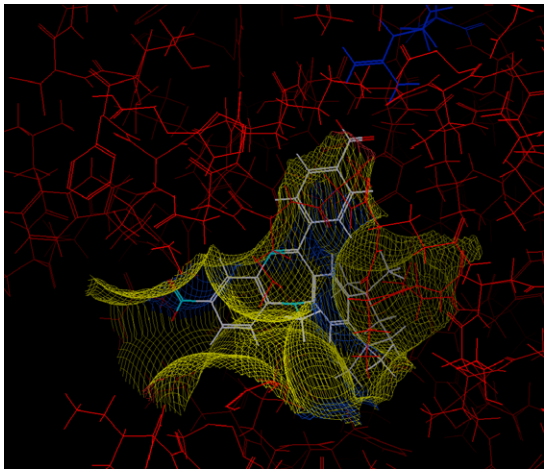

Figure 2. Docking of HX531 in a homology model of the antagonist-bound form of RXRα.

Keywords: RXR; Antagonist; Diazepinylbenzoic acid.
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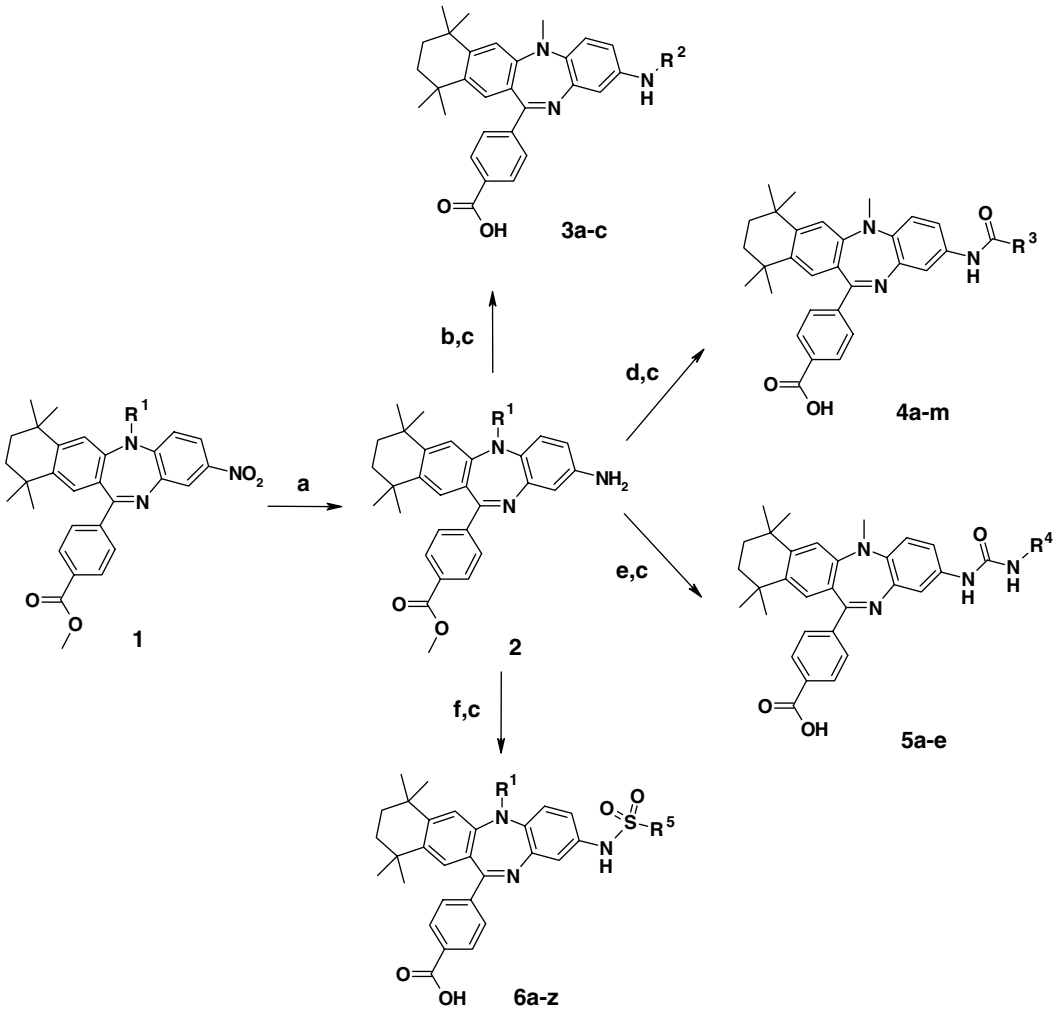

Scheme 1. Reagents and conditions: (a) $SnCl_2$–$2H_2O$, DMF, 60 °C; (b) i—$(Boc)_2O$, Et_3N, THF; ii—R^2X, NaH, DMF; iii—TFA, CH_2Cl_2; (c) 2 M NaOH, DMF; (d) R^3COX, Et_3N, CH_2Cl_2; (e) R^4NCO, Et_3N, CH_2Cl_2; (f) R^5SO_2Cl, Et_3N, CH_2Cl_2.

that appropriate functional antagonism of PPARγ/RXR may be a logical approach to protection against obesity and related diseases such as type 2 diabetes.

Thus, we initiated to seek more potent RXR antagonists with good pharmacokinetic properties based on the structure of HX531.

Figure 2 shows a docking of HX531 in a homology model of the antagonist-bound form of RXRα based on the X-ray structure of agonist-bound RXRα and antagonist-bound PPARα. On the basis of the structural information from the modeling study, we initially focused our attention of structural modification on two functionalized sites, the nitro group and the *N*-methyl group, shown in Figure 1 because an open molecular space around these two sites is available to accommodate structural modifications. More importantly, these two sites were found to be key determinants for antagonism and play an important role as agonist/antagonist switch.[5]

The common intermediate amine **2** was prepared by $SnCl_2$ reduction of the corresponding nitro **1** which was synthesized by the reported procedures.[5,7] The Boc protection of **2** followed by alkylation and deprotection afforded *N*-alkylamine derivatives **3a**–**c**. Amide **4a**–**m**, ureido **5a**–**e**, and sulfonamide **6a**–**z** analogues were synthesized by direct acylation of **2** with acid chloride, isocyanate, and sulfonyl chloride, respectively (Scheme 1).[8,9]

The diazepinylbenzoic acid derivatives possessing a variety of functional groups replacing the nitro group of HX531 were evaluated in the inhibition assay of the transactivation activity for RXRα homodimer using LG100268 as an RXR selective ligand and 9-*cis*-RA as a natural ligand.[10]

The amine derivatives **3** turned out to be inactive in the reporter gene assay although other analogues, the amides **4**, the ureidos **5**, and the sulfonamides **6**, were comparably active to HX531. It may suggest that an electron-withdrawing group seems to be essential as a functional group for the antagonistic activity. The amide derivatives **4** inhibited the transactivation with 9-*cis*-RA more strongly than with LG100268 whereas the ureidos **5** inhibited it with 9-*cis*-RA and LG100268 equally (Table 1).

Table 1. Inhibition of the transactivation of RXRα with amine derivatives (**3**–**5**)

Compound	**3**: R^2 **4**: R^3 **5**: R^4	LG[a] IC_{50} (μM)	9-RA[b] IC_{50} (μM)
HX531		1.2	0.9
3a	Me	>100	>10
3b	*n*-Bu	>100	>10
3c	Benzyl	>100	>10
4a	Et	7.1	2.2
4b	*n*-Pr	3.9	1.8
4c	*i*-Pr	5.6	1.9
4d	Cyclohexyl	4.5	1.5
4e	Adamantyl	7.2	2.9
4f	Phenyl	2.0	0.9
4g	4-F-phenyl	1.6	1.7
4h	3-CF_3-phenyl	4.1	4.2
4i	Benzyl	2.5	0.97
4j	4-F-benzyl(R^1 = Me)	1.3	0.88
4k	4-F-benzyl(R^1 = Et)	1.2	0.72
4l	4-F-benzyl(R^1 = Pr)	3.9	3.1
4m	Stylyl	5.2	3.8
5a	Me	14	15
5b	Et	7.4	7.3
5c	*n*-Pr	5.2	6.2
5d	*n*-Bu	3.4	3.3
5e	Benzyl	2.5	2.8

Values are means of three independent experiments measured in duplicate.
[a] LG100268.
[b] 9-*cis*-Retinoic acid.

Of these compounds, the sulfonamide analogues **6** were found to be most potent RXR antagonists (Table 2). Both simple alkyl and aryl sulfonamides were generally tolerable and show an antagonistic activity with the IC_{50} value below 1 μM. A propyl or butyl group is an optimum size as an alkyl sulfonamide substitution (R^5) and an ethyl group is a most preferable substituent for N-substitution (R^1). Accordingly, the compounds **6e** and **6g** having these combinations of alkyl substitution (**6e**: R^1 = Et, R^5 = Pr, **6g**: R^1 = Et, R^5 = Bu) exerted a high antagonistic activity against both LG100268 and 9-*cis*-RA.

In the case of aryl sulfonamides, the 4-F-substitution pattern (**6n**) is more active than the corresponding 2-F-(**6l**) or 3-F-substitution (**6m**). Thus, its *N*-ethyl substitution (**6o**) showed an IC_{50} value below 0.5 μM both for LG100268 and 9-*cis*-RA. The replacement of 4-F group of phenyl sulfonamide with a methyl or methoxy group resulted in a significant decrease in the antagonistic activity. It is intriguing that the 3-substitution pattern (**6t**) of the trifluoromethyl group on the phenyl group showed a good result. The replacement of the *N*-methyl group of **6t** with an ethyl (**6v**) or a propyl group (**6w**) gave best results, IC_{50} values of these compounds exerted below 0.1 μM when 9-*cis*-RA was used as a ligand. However, larger biaryl substituents (**6y** and **6z**) and even 3,5-bis-trifluoro-methyl phenyl group (**6x**) were unacceptable in this open space.

The pharmacokinetics profiles of **6v** and **6w** were evaluated after the intravenous (1 mg/kg bolus) and peroral

Table 2. Inhibition of the transactivation of RXRα with amine derivatives (**6**)

Compound	R^1	R^5	LG[a] IC_{50} (μM)	9-RA[b] IC_{50} (μM)
6a	Me	Me	4.4	2.4
6b	Me	Et	4.9	0.84
6c	Me	*n*-Pr	2.1	0.25
6d	Me	*n*-Bu	0.91	0.37
6e	Et	*n*-Pr	0.57	0.34
6f	*n*-Pr	*n*-Pr	0.84	0.55
6g	Et	*n*-Bu	0.32	0.2
6h	*n*-Pr	*n*-Bu	0.85	0.14
6i	Me	Benzyl	5.2	1.0
6j	Me	Stylyl	2.9	0.4
6k	Me	Phenyl	1.4	0.51
6l	Me	2-F-phenyl	4.4	2.3
6m	Me	3-F-phenyl	2.3	1.2
6n	Me	4-F-phenyl	1.7	0.19
6o	Et	4-F-phenyl	0.42	0.22
6p	*n*-Pr	4-F-phenyl	0.81	0.13
6q	Me	4-Me-phenyl	2.5	0.43
6r	Me	4-OMe-phenyl	2.5	0.47
6s	Me	2-CF_3-phenyl	2.6	1.4
6t	Me	3-CF_3-phenyl	1.5	0.48
6u	Me	4-CF_3-phenyl	2.2	1.1
6v	Et	3-CF_3-phenyl	0.16	0.095
6w	*n*-Pr	3-CF_3-phenyl	0.19	0.076
6x	Me	3,5-Bis-CF_3-Ph	4.1	1.2
6y	Me	1-Naphthyl	3.4	0.85
6z	Me	4-Biphenyl	6.2	1.3

Values are means of three independent experiments measured in duplicate.
[a] LG100268.
[b] 9-*cis*-Retinoic acid.

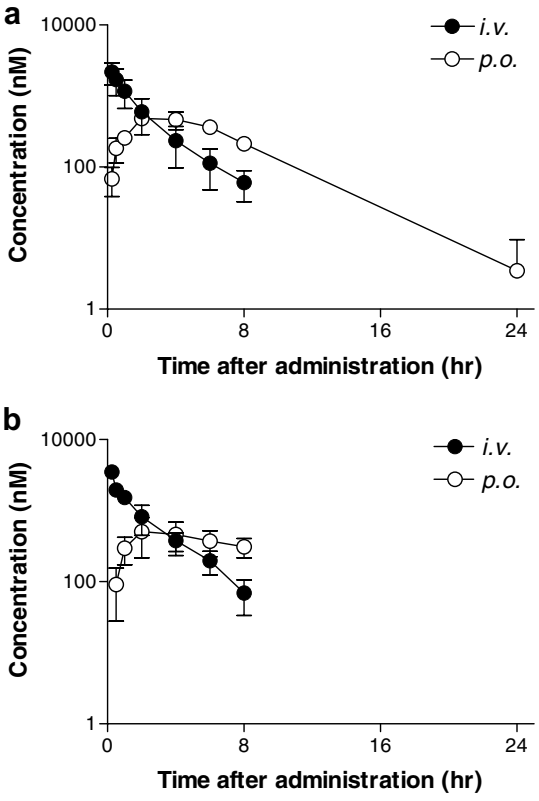

Figure 3. Plasma concentration–time profiles of **6v** (a) and **6w** (b) after intravenous (iv) and peroral (po) administration in rats.

(3 mg/kg as suspension) administration in rats (Fig. 3). Blood samples were taken at various time points after administration and blood levels of parent compounds were measured with an LC/MS method. After intravenous administration, **6v** and **6w** were cleared biphasically from plasma with the CL p values of 0.5 ± 0.2 and 0.4 ± 0.1 L/h/kg, and the terminal elimination half-lives were 2.1 ± 0.3 and 1.8 ± 0.7 h, respectively. The volumes of distribution of **6v** and **6w** at the steady state were calculated as 1.2 ± 0.5 and 1.1 ± 0.3 L/kg. After peroral administration, **6v** and **6w** were absorbed well as shown by the mean t_{max} of 4.7 ± 1.2 and 4.0 ± 3.5 h reaching the C_{max} of 468 ± 129 and 519 ± 270 nM, respectively. Oral bioavailabilities of **6v** and **6w** at doses 1 mg/kg for intravenous and 3 mg/kg for peroral dosing were 36 and 21%, respectively. These results suggested that these compounds seem to be a promising candidate for the indications requiring peroral administration.

In summary, we found potent RXR antagonists based on the diazepinylbenzoic acid scaffold. An antagonistic activity of the newly synthesized derivatives is dependent on a size of the *N*-alkyl group (R^1) and the side chain (R^2–R^5) of the functional groups on the diazepine phenyl group. It is also influenced by a degree of electron-withdrawing property of the functional group. The sulfonamide derivatives (**6v** and **6w**) reveal a high antagonistic activity and good pharmacokinetic properties. These compounds are promising oral agents for the treatment of diabetes and obesity.

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8. Synthesis of compound **6v**: To a solution of 5-ethyl-7,7,10,10-tetramethyl-7,8,9,10-tetrahydro-2-[3-(trifluoromethyl)phenylsulfonylamino]-5H-5,13-diazabenzo[4,5]-cyclohepta[1,2-*b*]naphthalene-12-yl)benzoic acid methyl ester (1.10 g, 0.91 mmol) in DMF (12 ml) was added 2 N NaOH (6.0 ml, 12 mmol) at room temperature. The reaction mixture was stirred at the same temperature overnight. The mixture was acidified with 1 N HCl, diluted with H_2O, and extracted with ether. The organic layer was washed twice with H_2O, dried over $MgSO_4$, and evaporated in vacuo. The resulting solid was washed with hexane/ether (1:1) and dried to give **6v** (400 mg, 65%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.06 (3H, s), 1.14 (3H, s), 1.21 (3H, t), 1.26 (3H, s), 1.31 (3H, s), 1.62–1.70 (4H, m), 3.53–3.72 (2H, m), 6.42 (1H, s), 6.84–6.88 (3H, m), 6.92–6.96 (2H, m), 7.59 (1H, t), 7.78 (1H, d), 7.86 (2H, d), 7.95–7.97 (3H, m), 8.13 (1H, d).
9. Compound **6w**: ^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.80 (3H, t), 1.01 (3H, s), 1.10 (3H, s), 1.23 (3H, t), 1.27 (3H, s), 1.44–1.62 (6H, m), 3.37–3.43 (1H, m), 3.65–3.70 (1H, m), 6.84–6.99 (5H, m), 7.73 (2H, d), 7.82 (1H, t), 7.90 (1H, s), 7.99–8.07 (4H, m), 10.29 (1H, br s), 13.14 (1H, br s).
10. EK-293 cells were co-transfected with the hRXRα expression vector, pcDNA-hRXRα and the reporter plasmid, pGL3(CRBPII)$_2$. For transfection, 100 μL/well of the cell suspension (1.2×10^6 cells/mL) was mixed with DNA-LipofectAmine2000 (50 μL/well in OPTI-MEM I serum free medium) and dispensed to the wells of a 96-well flat-bottomed assay plate. After a 6-h incubation, 2 nM LG100268 or 20 nM 9-*cis*-RA as the activating ligands was added in the presence or absence of a test compound $\alpha\tau$ 0.001 τo 10 μM (final concentration) as dilutions of 50 μL/well of the medium. After further 20 h incubation, the cells were lysed and the luciferase activity was measured.