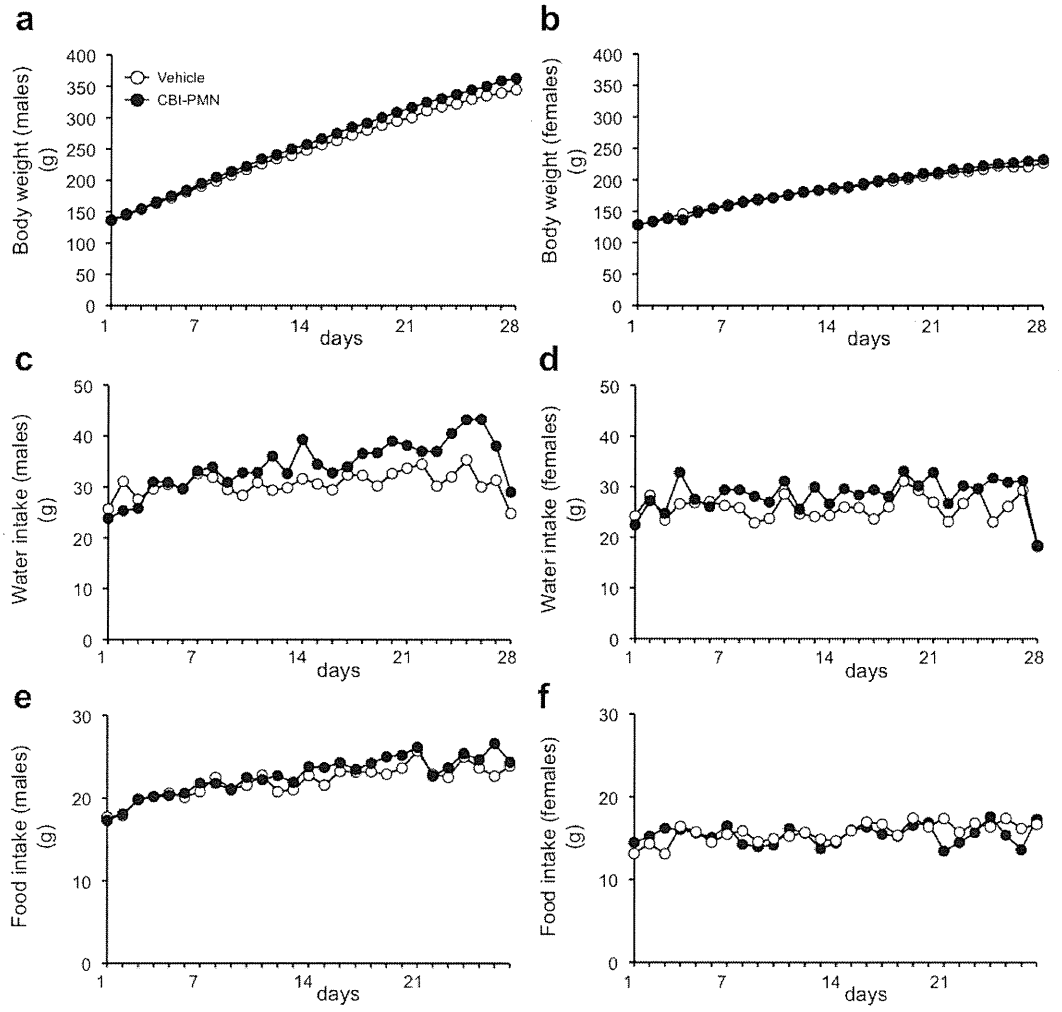


Figure 3. CBt-PMN ラット 28 日間反復経口投与による体重推移，飲水量，摂餌量．薬物非投与群には 0.5% CMC 水溶液を，CBt-PMN 投与群は 30 mg/kg/day で投薬した．**a–b)** 体重推移．**c–d)** 摂餌量．**e–f)** 飲水量．オス：**a**, **c**, および **e**．メス：**b**, **d**, および **f**．○：vehicle，●：CBt-PMN．n = 6.

Table 1. CBt-PMN 30 mg/kg/day 28 日間薬物経口反復経口投与による各臓器の重量

	Males		Females	
	Vehicle	CBt-PMN	Vehicle	CBt-PMN
Weight (g)	324.3 ± 8.7	337.8 ± 4.6	213.4 ± 2.1	216.1 ± 10.7
Brain (g)	2.0 ± 0.1	2.0 ± 0.1	1.8 ± 0.0	1.7 ± 0.0
Liver (g)	9.7 ± 0.6	9.7 ± 0.4	5.9 ± 0.2	6.2 ± 0.4
Kidney (g)	2.5 ± 0.1	2.6 ± 0.1	1.6 ± 0.0	1.5 ± 0.1
Spleen (g)	0.6 ± 0.0	0.7 ± 0.1	0.4 ± 0.0	0.5 ± 0.0
Testis (g)	3.9 ± 0.2	4.5 ± 0.1 *		

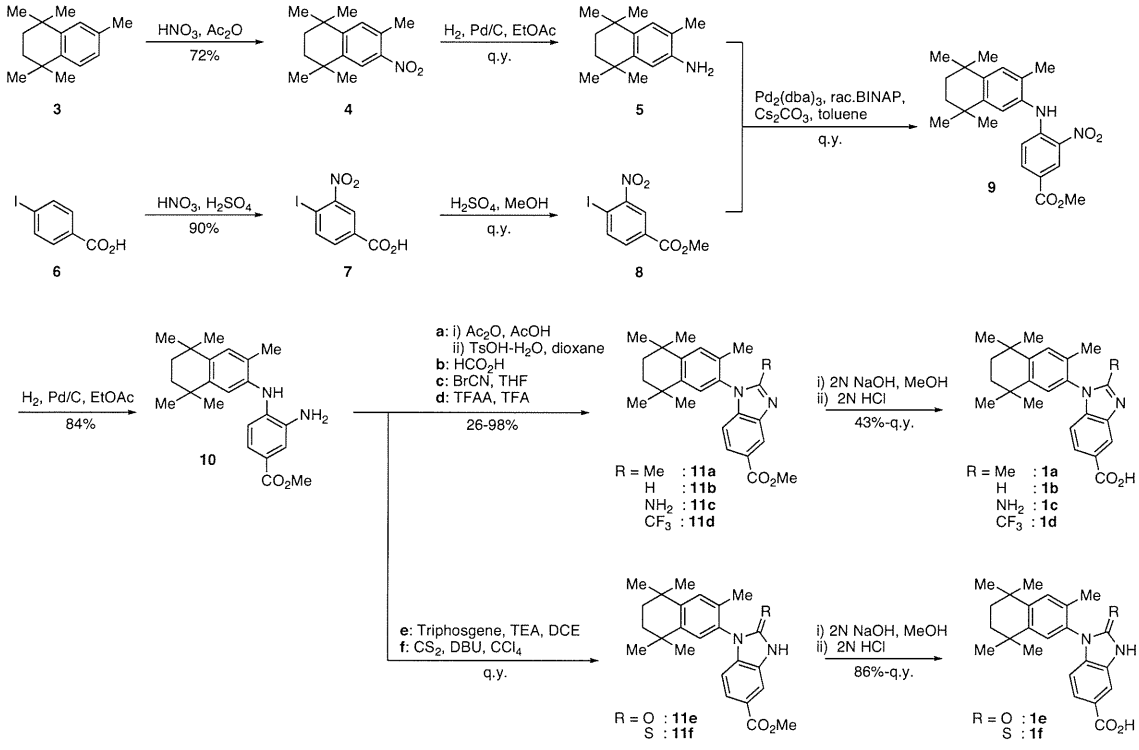
データ は平均 ± s.e.m. ; 有意差: * $p < 0.05$ vs. vehicle, n = 6.

Table 2. CBt-PMN 30 mg/kg/day でのラット 28 日間薬物経口反復経口投与による血液生化学データ

	Males			Females		
	vehicle	CBt-PMN	Reference[c]	vehicle	CBt-PMN	Reference[c]
WBC ($\times 10^2$/mL)[a]	71.7 ± 4.2	92.5 ± 18.6	–	76.5 ± 9.3	72.5 ± 7.5	–
RBC ($\times 10^4$/mL)[a]	741.8 ± 17.4	746.2 ± 11.7	–	778.3 ± 18.1	767.3 ± 12.0	–
PLT ($\times 10^4$/mL)[a]	123.3 ± 5.7	139.9 ± 13.1	–	129.3 ± 4.9	140.3 ± 7.1	–
HGB (g/dL)[a]	14.8 ± 0.2	14.4 ± 0.2	14.4 – 16.0	14.7 ± 0.2	14.5 ± 0.2	13.7 – 15.7
HCT (%)[a]	45.5 ± 0.6	45.0 ± 0.7	41.2 – 47.3	45.7 ± 0.8	44.8 ± 0.6	9.6 – 46.0
MCV (fL)[a]	61.4 ± 0.8	60.4 ± 0.4	53.0 – 59.5	58.7 ± 0.6	58.4 ± 0.6	53.6 – 58.1
MCH (pg)[a]	20.0 ± 0.2	19.3 ± 0.2 *	18.3 – 20.0	18.9 ± 0.3	18.9 ± 0.2	18.6 – 20.0
MCHC (g/dL)[a]	32.5 ± 0.1	31.9 ± 0.2 *	32.7 – 35.7	32.2 ± 0.3	32.2 ± 0.1	32.8 – 36.2
AST (U/I)[b]	65.7 ± 3.5	75.2 ± 2.8 *	87.0 – 114.0	60.2 ± 1.6	64.8 ± 2.0 *	85.0 – 123.0
ALT (U/I)[b]	27.8 ± 1.4	37.7 ± 2.0 **	28.0 – 40.0	21.5 ± 2.1	29.3 ± 1.9 **	25.0 – 36.0
γ-GTP (U/I)[b]	8.2 ± 0.4	8.3 ± 0.2	0.0 – 1.0	8.3 ± 0.2	8.7 ± 0.3	0.0 – 0.4
ALP (U/I)[b]	585.7 ± 34.3	607.5 ± 39.7	–	434.3 ± 50.7	476.5 ± 75.2	–
CRE (mg/dL)[b]	0.2 ± 0.0	0.2 ± 0.0	0.5 – 0.6	0.3 ± 0.0	0.3 ± 0.0	0.5 – 0.6
BUN (mg/dL)[b]	16.4 ± 0.7	11.8 ± 0.4 **	13.0 – 16.0	13.8 ± 0.7	14.5 ± 0.9	11.0 – 16.0
TG (mg/dL)[b]	44.8 ± 2.8	50.8 ± 2.9	61.0 – 99.0	55.0 ± 4.9	77.5 ± 5.5 **	42.0 – 74.0
TCHO (mg/dL)[b]	56.2 ± 7.5	34.0 ± 4.5 *	54.0 – 74.0	25.7 ± 3.7	44.8 ± 10.6	67.0 – 87.0
GLU (mg/dL)[b]	181.2 ± 16.1	149.8 ± 17.7	112.0 – 176.0	118.3 ± 5.7	145.5 ± 10.6 *	113.0 – 185.0

a. 測定は pocH-100i (Sysmex)を用いた.
b. 測定は Fuji Dry Chem 4000V (Fuji Medical Co., Tokyo, Japan) を用いた.
c. Charles River®より供給されている Crl:CD(SD) Rats の Clinical Laboratory Parameters (CRL_Mar,2006) .
データ は平均 ± s.e.m. ; 有意差: * $p < 0.05$ vs. vehicle. ** $p < 0.01$ vs. vehicle.

Scheme 1



Scheme 2

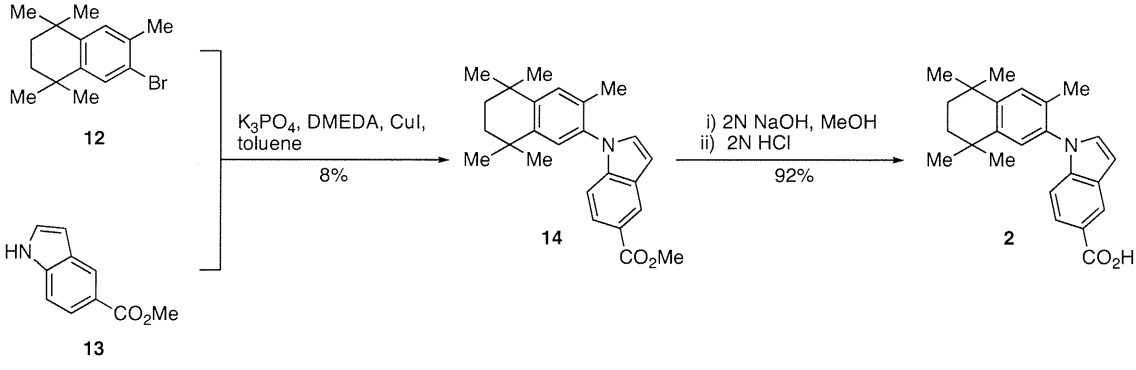
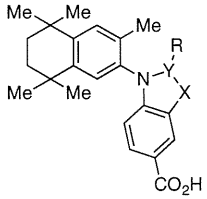


Table 3. 本研究で新たに創出した化合物の RXR 転写活性化能



			RXRα[a]			
Compound	X	Y-R	EC_{50} (nM)[b]	E_{max} (%)	Efficacy at 1 μM (%)	Efficacy at 10 μM (%)
NEt-TMN	–	–	3.8 ± 0.2	96 ± 4	96 ± 4	98 ± 3
CBt-PMN	N	N	143 ± 2	75 ± 4	75 ± 3	69 ± 3
1a	N	C-CH_3	367 ± 130	95 ±5	70 ± 4	80 ± 5
1b	N	C-H	633 ± 33	75 ± 3	52 ± 3	72 ± 4
1c	N	C-NH_2	155 ± 10	103 ± 2	35 ± 3	96 ± 4
1d	N	C-CF_3	15 ± 0	67 ± 2	71 ± 6	66 ± 3
1e	NH	C=O	n.d.	i.a.	1 ± 0	22 ± 2
1f	NH	C=S	n.d.	i.a.	2 ± 0	37 ± 0
2	CH	C-H	83 ± 8	73 ± 2	67 ± 5	77 ± 4

a) All values represent the mean value of at least two separate experiments with triplicate determinations. Luciferase activity of LGD1069 (RXR full agonist) at 1 μM was defined as 100 percent.

b) EC_{50} values were determined from full dose-response curves in COS-1 cells.

Table 4. 本研究で新たに創出した化合物の RXR 転写活性化能（図は Table 3 に同じ）

			RXRα[a]		Properties of R		
Compound	X	Y-R	EC_{50} (nM)	E_{max} (%)	σ[b]	Es[c]	π[d]
CBt-PMN	N	N	143 ± 2	75 ± 4	–	–	–
1a	N	C-CH_3	367 ± 130	95 ±5	-0.17	-1.24	0.56
1b	N	C-H	633 ± 33	75 ± 3	0.00	0.00	0.00
1c	N	C-NH_2	155 ± 10	103 ± 2	-0.66	-0.61	-1.23
1d	N	C-CF_3	15 ± 0	67 ± 2	0.54	-2.40	0.88

a) all data are the same as described in Table 3. b) σ: electronic effect, c) Es: Taft's steric substituent constants (steric effect), d) π: lipophilicity. These data were cited from Hansch, C., Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology,* Wiley-Interscience, NY, 1979.

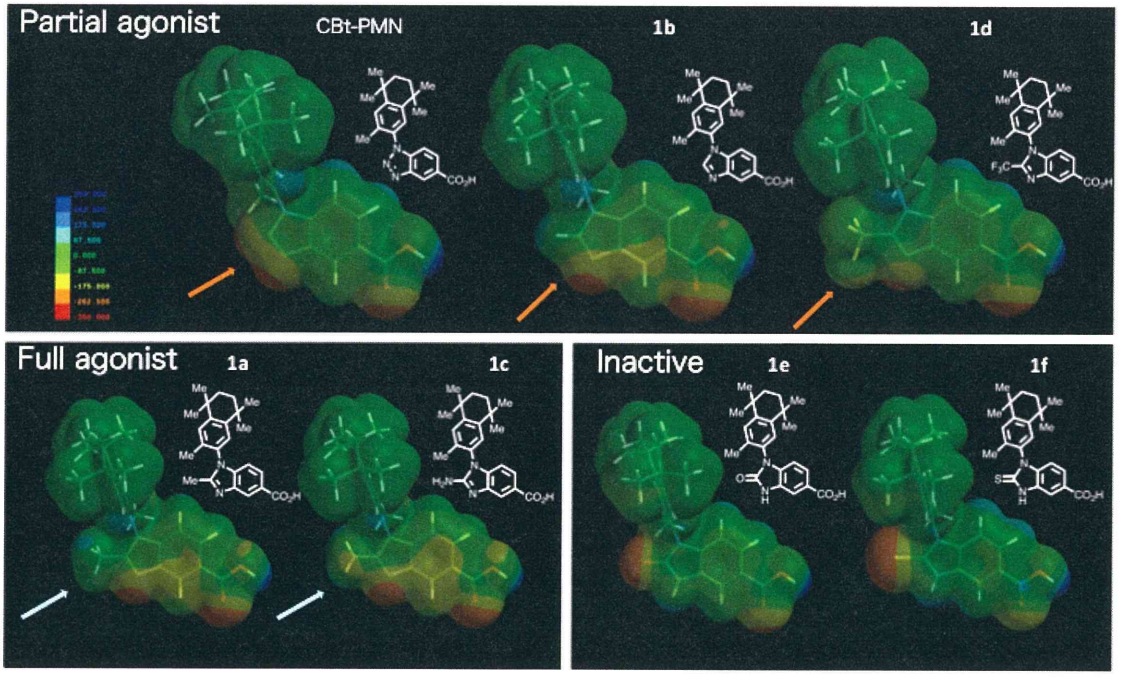


Figure 4. CBt-PMN および創出化合物の分子表面静電ポテンシャル図．フルアゴニスト活性を示した化合物は，ともに→で示す部分が弱い正の静電ポテンシャルを示し，RXR パーシャルアゴニスト活性を示した化合物は，→で示す部分に弱い負の静電ポテンシャル部位が存在することが示唆された．

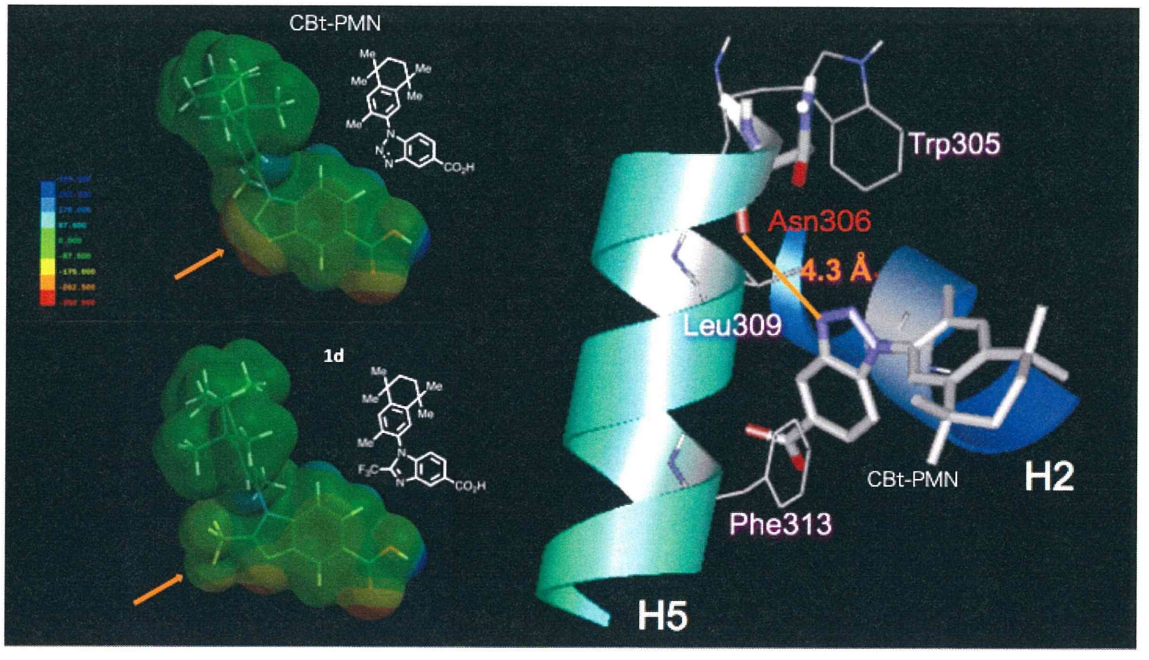


Figure 5. CBt-PMN および **1d** の分子表面静電ポテンシャル図と RXR のリガンド結合部位における CBt-PMN のドッキング図．閉環部位に見られる弱い負の静電ポテンシャル部分と RXR ヘリックス 5 上の Asn306 との静電反発が示唆された．

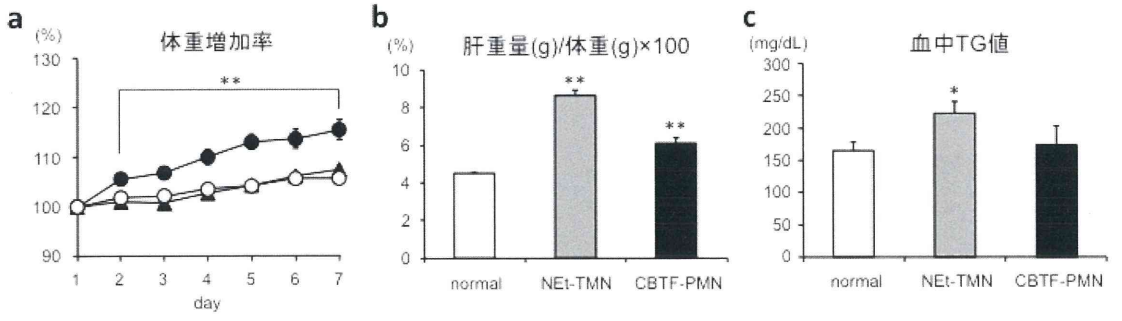


Figure 6. RXRアゴニストの雄性ICRマウス7日間連続経口投与による体重推移ならびに肝肥大. 薬物はいずれも，30 mg/kg/day で経口投与した．a) 体重推移．○：薬物非投与，●：NEt-TMN，▲：**1d** (CBTF-PMN). b) 薬物 7 日投与後（投薬開始 7 日後）の肝臓重量，c) 血中トリグリセリド（TG）値．データ は平均 ± s.e.m.；有意差: * $p < 0.05$ vs. vehicle. n = 8.

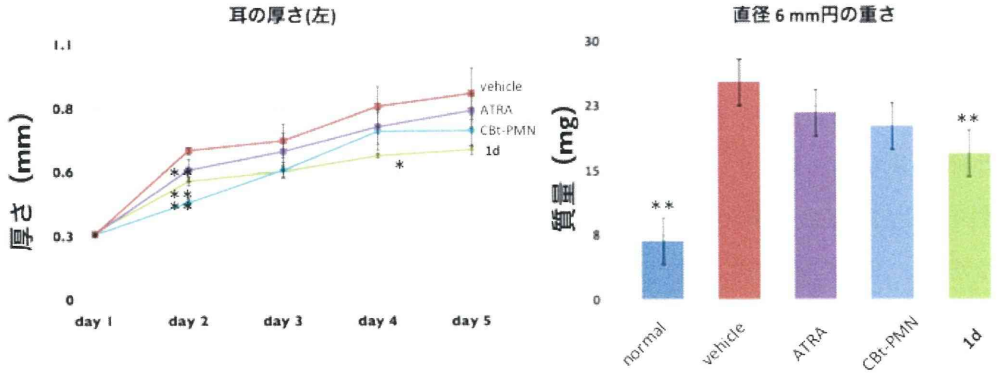


Figure 7. TPA 誘発皮膚炎モデルマウスの耳の厚さ変化ならびに直径 6 mm 円での耳重量. 左) 耳の厚さ変化．Vehicle: 薬物非投与群，ATRA: all-*trans* retinoic acid 5 mM in acetone 塗布群，CBt: CBt-PMN 5 mM in acetone 塗布群，および **1d** 5 mM in acetone 塗布群. 右) day5 における直径 6 mm 円での耳重量. Normal: 非炎症群，vehicle: 炎症・非薬物投与群，ATRA: all-*trans* retinoic acid 5 mM in acetone 塗布群，CBt: CBt-PMN 5 mM in acetone 塗布群，および **1d** 5 mM in acetone 塗布群．データ は平均 ± s.e.m.；有意差: * $p < 0.05$ vs. vehicle. n = 5.

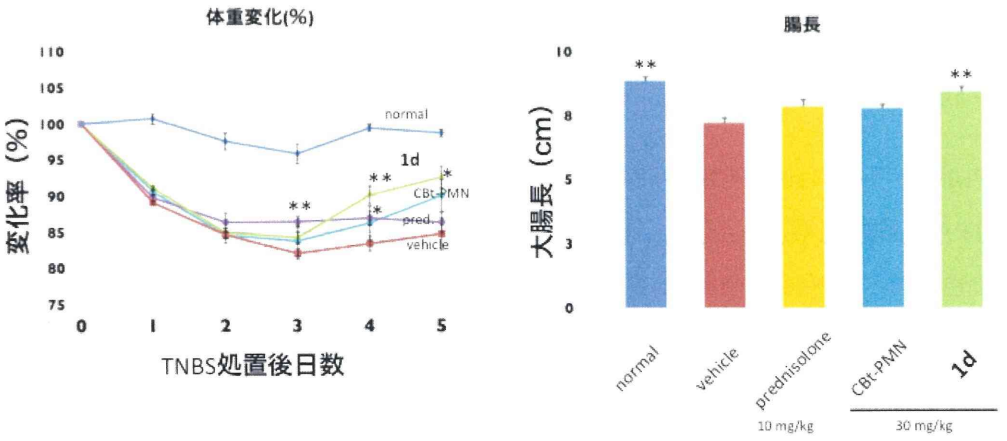


Figure 8. TNBS 誘発腸炎モデルマウスの体重推移（変化率）．TNBS 注腸時を 0 日目として示してある．Normal: 非炎症群，vehicle: 炎症・非薬物投与群，pred: prednisolone 10 mg/kg/day 投与群，CBt: CBt-PMN 30 mg/kg/day 投与群，**1d**: **1d** 30 mg/kg/day 投与群．データ は平均 ± s.e.m.；有意差: * $p < 0.05$ vs. vehicle. n = 5.

D. 考察

CBt-PMNはラットを用いた長期連投により，30 mg/kg/dayでの経口投与であれば，RXRフルアゴニストに見られる体重増加や肝肥大を生じないことが示された．生化学的血液検査では，薬物非投与群との差異が認められたものがあるものの，動物供給業者から提供されている正常値範囲内であることから，この投与量であれば，副作用発現は極めて生じにくいことが示された．

CBt-PMNがRXRパーシャルアゴニスト活性を示す要因について調べたところ，閉環構造に加え，閉環部位2位に弱い負の静電ポテンシャルを有することによることが示唆された．さらにこの部位は，RXRのHelix5上のAsn306と近接していることから，RXRパーシャルアゴニスト活性はRXRのAsn306との相互作用により生じていることが推測された．さらに本研究を通じて，CBt-PMNよりも低濃度で効果の見られるRXR パーシャルアゴニスト**1d**（CBTF-PMN）の創出に至った．

CBt-PMNならびに**1d**（CBTF-PMN）を用いたTPA乾癬様皮膚炎モデル，またTNBS誘発クローン病様モデルマウスを用いた実験により，CBt-PMNの薬効は極めて弱い一方で，**1d**に顕著な薬効が認められた．なお，作用メカニズム解明を目的とするPCR解析は平成23年度以内には終了しておらず，平成24年度に引き続き実施する予定である．また，研究分担社によりリウマチモデルの作成ならびにプレドニゾロンでの薬効評価も行えたことから，平成24年度には，**1d**について抗リウマチ作用を調べる予定である．

E. 結論

CBt-PMNは長期投与で副作用がみられないものの，本試験で評価したTPA誘発皮膚炎，またTNBS誘発クローン病モデルでは顕著な薬効を示さないことが分かった．一方で，CBt-PMNのRXRパーシャルアゴニスト活性を示すメカニズム研究を通じ，CBt-PMNより低濃度でRXR活性化能を示す新たなRXRパーシャルアゴニスト**1d**（CBTF-PMN）の創出に成功し，このデータを踏まえ，特許出願に至った．

平成 24 年度には，本化合物の長期投与による副作用発現，**1d** の抗リウマチ作用や作用メカニズム解明に向けた遺伝子発現解析について実施する．今後これらのデータも含めて，RXR パーシャルアゴニストが，RXR フルアゴニストに見られる体重増加や肝肥大，血中トリグリセリドの上昇を回避しつつ，免疫疾患モデル動物において有益な薬効を示すデータを得，製薬企業等との共同研究へとつなげて行きたい．

F. 研究発表

1．論文発表

Kakuta H, Yakushiji N, Shinozaki R, Ohsawa F, Yamada S, Ohta Y, Kawata K, Nakayama M, Hagaya M, Fujiwara C, Makishima M, Uno S, Tai A, Maehara A, Nakayama M, Oohashi T, Yasui H, Yoshikawa Y. RXR partial agonist CBt-PMN exerts therapeutic effects on type 2 diabetes without the side effects of RXR full agonists. *ACS Med. Chem. Lett.*, 2012, *3*, 427–432.

2．学会発表

大澤 史宜，薬師寺 信匡，篠崎 亮介，山田 翔也，中山 真理子，川田 浩平，槇島 誠，加来田 博貴，RXRアゴニストCBt-PMNのパーシャルアゴニスト活性発現機構の解明，日本薬学会第132年会（札幌）

G. 知的所有権の取得状況

名称：RXRパーシャルアゴニスト，特願2012-041353，出願日：平成24年2月28日，発明者：加来田博貴，出願人：岡山大学

II. 分担研究報告

厚生労働科学研究費補助金　（創薬基盤推進研究事業）
分担研究報告

リウマチモデルの作成と評価系の確立

研究分担者　大橋俊孝

岡山大学大学院医歯薬学総合研究科　分子医化学　准教授

研究要旨

本研究では，研究代表者により創出されるRXRパーシャルアゴニストによる抗リウマチ作用を評価するために，リウマチモデルの作成と薬効評価系を確立した．コラーゲン抗体誘発関節炎モデルマウスを，II型コラーゲン上の複数の関節炎惹起エピトープを認識する5種のモノクローナル抗体の混合液をChondrex®より購入し，重篤な関節炎を惹起することが知られるDBA/1マウスに投与することで作成した．また，薬効評価系の確立を目的に，ステロイド系抗炎症剤であるプレドニゾロンを10 mg/kg/dayで経口投与した．その結果，本モデルにおけるリウマチモデルの構築さらにプレドニゾロンの薬効が確認され，創出化合物の抗リウマチ作用を評価する系として利用可能であることが確認された．

A.　研究目的

研究代表者により創出されるRXRパーシャルアゴニストの抗リウマチ作用を評価するために，抗リウマチモデルの作成および薬効評価系の確立を目指した．

B.　研究方法

1）モデルの作成と病態評価

実験動物は7週齢の雌のDBA/1Jマウスを用いた．まず，体重による群分けを行い，関節炎惹起カクテル Arthrogen-CIA Arthritogenic Monoclonal Antibody (Chondrex®)　(10 mg/mL)を150 μL腹腔内注射した（0日）．3日目にLPS(0.5 mg/mL)を100 mL腹腔内注射した．臨床症状は発赤・腫脹の症状をもとにスコア化を行った（Minaguchi et al., 2008）．1足あたり最高点4点とし，4足分16点満点とする．通常4日目あたりから，発赤を認め，腫脹のピークは7日目にみられる．5日目に手足の腫脹が適度に見られたため，薬物（prednisolone）を10 mg/kgの投与量で投与を開始した．その後14日目まで手足を観察し，Table 1に示す基準（スコア）を用いてスコア化を行うことで評

価した.

Table 1. コラーゲン抗体誘発マウスリウマチモデルで利用した炎症判定スコア

0	Normal
1	Mild but definite redness and swelling of the ankle or wrist, or redness and swelling of any severity for 1 digit
2	Moderate to severe redness and swelling of the ankle or wrist
3	Redness and swelling of the entire foot including digits
4	Maximally inflamed limb with involvement of multiple joints

（倫理面への配慮）

いずれの動物実験とも，岡山大学動物実験委員会の承認を得て実施した.

2）統計学的処理

有意差検定は vehicle 群と薬物投与 群との間は， t 検定法（F 検定を含む）により行った.

C. 研究結果

Figure 1に，炎症スコアの結果を示す. prednisoloneの薬効評価を行ったところ，10 mg/kg/dayでの薬効を示せたことから，創出化合物の薬効評価を行うモデルとして利用可能であると判断された.

D. 考察

創出した抗リウマチモデルにおいて，ステロイド系抗炎症剤であるprednisoloneが10 mg/kg/dayでの経口投与により，顕著な抗リウマチ作用が認められたことから，本モデルを用いることで，創出されたRXRパーシャルアゴニストの抗リウマチ作用が評価可能と判断される.

H. 結論

抗リウマチモデルを作成し，さらに薬物投与を本実験で用いたprednisoloneと同様に投薬計画を行うことで，創出化合物の抗リウマチ作用の評価が可能である.

I. 研究発表

なし

J. 知的所有権の取得状況

なし

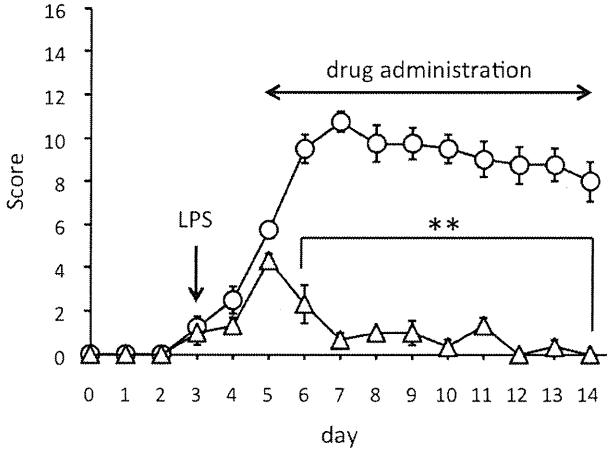


Figure 1. コラーゲン抗体誘発リウマチモデルマウスの炎症スコア．○：vehicle，△：prednisolone 10 mg/kg/day 経口投与群．データ は平均 ± s.e.m.；有意差: * $p < 0.05$ vs. vehicle. n = 5.

厚生労働科学研究費補助金　（創薬基盤推進研究事業）
分担研究報告

薬効メカニズム解明を目的とする遺伝子等発現解析

研究分担者　大塚正人

岡山大学自然生命科学研究支援センター　准教授

研究要旨

本研究では，研究代表者により創出されるRXRパーシャルアゴニストによるクローン病治療効果，抗リウマチ作用における薬効メカニズム解明を目的に，評価に用いた動物組織をもとに，PCRによるサイトカイン等の発現を解析することを目的とした．なお，TNBS誘発クローン病モデル，TPAによる皮膚炎モデルでの薬効評価が平成24年初頭になったこと，さらに抗リウマチモデルにおける薬効評価が行えなかったため，平成23年度においては実施途中であり，平成24年度引き続き実施する．

A.　研究目的

研究代表者により創出されるRXRパーシャルアゴニストの免疫疾患モデルにおける薬効メカニズム解析を目的に，評価に用いた動物組織をもとに，PCRによるサイトカイン等の発現を解析する．

B.　研究方法

TNBS腸炎モデルマウスにおいては，エーテル麻酔下にて大腸を摘出し，大腸長を測定した後，ただちに大腸の中央部を5-10 mm程度摘出し，液体窒素にて保存した．

TPA誘発皮膚炎モデルについては，エーテルの深麻酔により安楽死させたマウスより炎症部位の耳を摘出し，トレパンにてサンプリングした部位以外を液体窒素にして保存した．

リウマチモデルについては，膝関節を切除した後，軟骨部位を摘出し，液体窒素にて保存した方法を予定している．

液体窒素保存されたサンプルの一部をTrisolに加えた後，ホモジナイザーによる破砕後，mRNA抽出，さらにcDNA化を行った．

PCR用プライマーは，Takaraより購入した．購入したプライマーの塩基配列情報をTable 1に掲載する．

C.　研究結果

現在，プライマーを用いたPCR条件等の検討を行っている．

D. 考察

引き続き，創出化合物の薬効メカニズム解析へとつなげる．

K. 結論

平成23年度はPCRによる薬効メカニズム解析は進行途中であり，平成24年度に引き続けて実施する．なお，本研究分担者は平成24年4月より摂南大学へ異動のため，以降については研究代表者ならびに研究分担者である大橋が実施する．

L. 研究発表

なし

M. 知的所有権の取得状況

なし

Table 1. 本研究で用いた primer 情報

Primer		Sequence
IL1β	Forward	5'-TCCAGGATGAGGACATGAGCAC-3'
	Reverse	5'-GAACGTCACACACCAGCAGGTTA-3'
IL2	Forward	5'-CCCAGGATGCTCACCTTCA-3'
	Reverse	5'-CCGCAGAGGTCCAAGTTCA-3'
IL4	Forward	5'-ACGGAGATGGATGTGCCAAAC-3'
	Reverse	5'-AGCACCTTGGAAGCCCTACAGA-3'
IL6	Forward	5'- CCACTTCACAAGTCGGAGGCTTA-3'
	Reverse	5'- CCAGTTTGGTAGCATCCATCATTTC-3'
IL10	Forward	5'-GCCAGAGCCACATGCTCCTA-3'
	Reverse	5'-GATAAGGCTTGGCAACCCAAGTAA-3'
IL17A	Forward	5'-CTGATCAGGACGCGCAAAC-3'
	Reverse	5'-TCGCTGCTGCCTTCACTGTA-3'
IL23	Forward	5'-ACATGCACCAGCGGGACATA-3'
	Reverse	5'-CTTTGAAGATGTCAGAGTCAAGCAG-3'
Foxp3	Forward	5'-TGCCTTCAGACGAGACTTGGA-3'
	Reverse	5'-GGCATTGGGTTCTTGTCAGAG-3'
IFNβ1	Forward	5'-CCTGGAGCAGCTGAATGGAA-3'
	Reverse	5'-TGGATGGCAAAGGCAGTGTAA-3'
TGFβ	Forward	5'-GTGTGGAGCAACATGTGGAACTCTA-3'
	Reverse	5'-CGCTGAATCGAAAGCCCTGTA-3'
TNFα	Forward	5'-TATGGCCCAGACCCTCACA-3'
	Reverse	5'-GGAGTAGACAAGGTACAACCCATC-3'

III. 研究成果を含む刊行物

研究成果の刊行に関する一覧表

雑誌

著者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hiroki Kakuta, Nobumasa Yakushiji, Ryosuke Shinozaki, Fuminori Ohsawa, Shoya Yamada, Yui Ohta, Kohei Kawata, Mariko Nakayama, Manabu Hagaya, Chisa Fujiwara, Makoto Makishima, Shigeyuki Uno, Akihiro Tai, Ami Maehara, Masaru Nakayama, Toshitaka Oohashi, Hiroyuki Yasui, N Yutaka Yoshikawa	RXR Partial Agonist CBt-PMN Exerts Therapeutic Effects on Type 2 Diabetes without the Side Effects of RXR Full Agonists	ACS Medicinal Chemistry Letters	3	427-432	2012

上記論文には，本事業にて行ったRXRパーシャルアゴニストCBt-PMNのラット長期投与データを掲載している．なお，掲載箇所はSupporting informationの**Figure S6**，**Table S3**および**Table S4**である．次頁より掲載物をそのまま掲載する．

ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

RXR Partial Agonist CBt-PMN Exerts Therapeutic Effects on Type 2 Diabetes without the Side Effects of RXR Full Agonists

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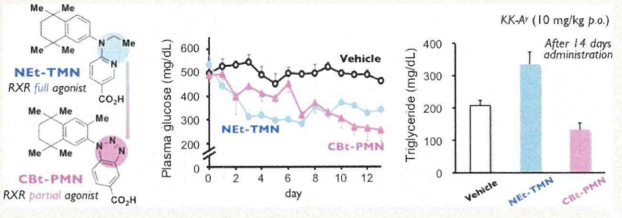
S *Supporting Information*

ABSTRACT: Treating insulin resistance and type 2 diabetes in rodents, currently known retinoid X receptor (RXR) agonists induce significant adverse effects. Here we introduce a novel RXR partial agonist CBt-PMN (**11b**), which shows a potent glucose-lowering effect and improvements of insulin secretion and glucose tolerance without the serious adverse effects caused by RXR full agonists. We suggest that RXR partial agonists may be a new class of antitype 2 diabetes drug candidates.



KEYWORDS: *Nuclear receptors, RXR, partial agonists, type 2 diabetes*

Retinoid X receptors (RXRs) are of interest as nuclear receptors that serve to regulate transcription of genes relevant to diabetes. They function either as homodimers or as heterodimers, for example with peroxisome proliferator-activated receptors (such as PPARγ, the well-known target of thiazolidinedione-type agents to improve insulin resistance) or liver X receptors (LXRs, whose activation induces glucose metabolism and improves glucose tolerance).[1−5] In addition, LXR activation is reported to induce glucokinase expression, which is coregulated with insulin,[6] and to promote insulin secretion via stimulation of pancreatic islets.[7] So-called permissive RXR-heterodimers, such as PPAR/RXR and LXR/RXR, can be activated by RXR agonists alone.[8] Therefore, RXR agonists seem to be promising candidates for improving both insulin resistance and glucose tolerance. Several RXR agonists **1**−**4** (Figure 1) have been evaluated for the treatment of insulin resistance and type 2 diabetes in rodents.[9] However, all of them induce significant adverse effects, such as blood triglyceride (TG) elevation,[10] weight gain,[11] hepatomegaly,[12] and hypothyroidism.[13]

On the basis of a report that structurally different RXR agonists show different patterns of activation of RXR-heterodimers,[14] we previously examined the feasibility of separating the blood glucose-lowering action of RXR agonists from the adverse effects. For this purpose, we administered RXR agonists NEt-TMN (**5**),[15] NEt-3IB (**6a**), and NEt-3IP (**6b**)[16] (Figure 1), which similarly activate RXR, but differently activate PPAR/RXR and LXR/RXR,[17] to KK-A^y type 2 diabetes model mice, and we examined the relationship of the RXR-heterodimeric activation pattern to the antihyperglycemic effect and adverse effects such as hepatomegaly and TG elevation.[18] We found that **6a** has a less potent TG-elevating activity and hepatomegaly than the other RXR agonists examined, though all of them showed similar blood glucose-lowering action on repeated administration. This result suggested that it might be possible to separate the therapeutic effects from the side effects of RXR agonists. However, even **6a** shows significant TG-elevation and hepatomegaly. Since all RXR agonists that have been reported to show serious side effects are RXR full agonists that potently activate RXR, we hypothesized that there is a

Received: March 2, 2012
Accepted: April 8, 2012
Published: April 9, 2012

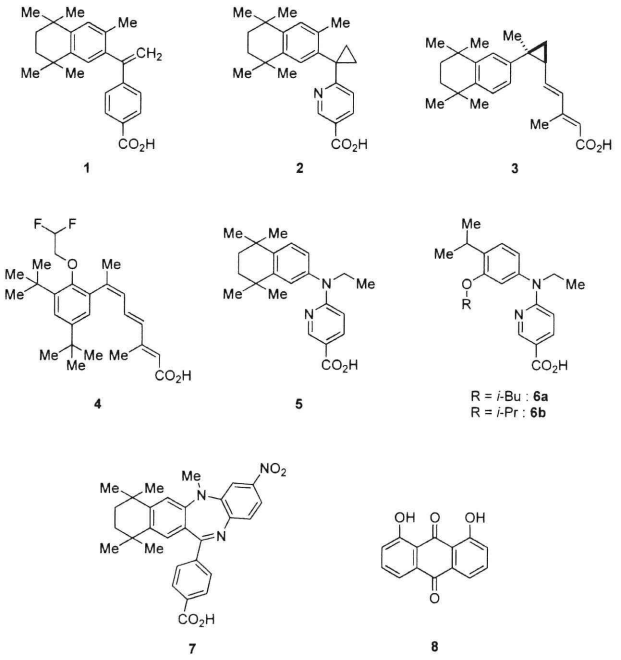


Figure 1. Chemical structures of known RXR ligands.

threshold difference between the therapeutic effects and adverse effects of RXR activation. Therefore, we decided to focus on RXR partial agonists, which would activate RXR only moderately or whose transcriptional efficacy would be limited. We further considered that this idea might be consistent with reports that RXR antagonists HX531 (**7**)[19] and danthron (**8**)[20] (Figure 1) show blood glucose-lowering effects in type 2 diabetes model mice, because partial antagonists are also thought to behave as partial agonists.

To test our hypothesis, we aimed to synthesize RXR partial agonists by modifying the representative RXR agonist structure, focusing on restriction of the molecular flexibility by linking the hydrophobic or acidic domain and the linking domains to form a new ring moiety (Figure 2). Screening of the synthesized

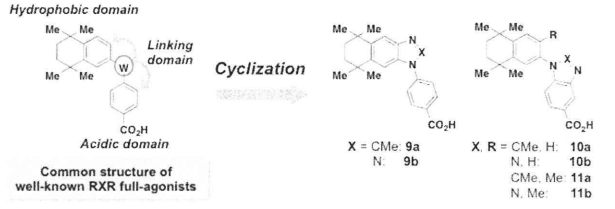


Figure 2. Molecular design strategy for creating RXR partial agonists.

compounds identified CBt-PMN (**11b**) as a novel RXR partial agonist. Studies in mice and rats showed that **11b** did not induce the side effects typically caused by RXR full agonists, while oral administration to KK-A^{y} mice, a widely used model of type 2 diabetes, resulted in a potent glucose-lowering effect and improved insulin secretion and glucose tolerance. These results indicate that RXR partial agonists may be a new class of antitype 2 diabetes drug candidates without the serious adverse effects shown by RXR full agonists. In this article, we describe the molecular design strategy and the results of the in vitro and in vivo experiments, and we discuss the mechanism of action of **11b**.

In general, representative RXR agonists consist of a hydrophobic domain including 1,1,4,4-tetramethyltetralin, an acidic domain bearing benzoic acid, nicotinic acid, or pyrimidinecarboxylic acid, and a linking domain connecting these domains, as shown in Figure 2. Our design strategy to

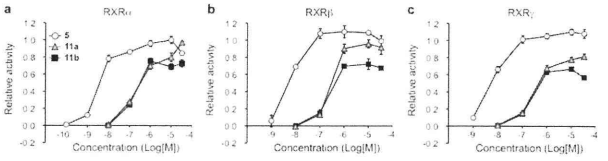


Figure 3. Results of reporter gene assays of **5**, **11a**, and **11b**. COS-1 cells were transfected with three kinds of vectors consisting of a RXR receptor subtype, a luciferase reporter gene under the control of the appropriate RXR response element (CRBPII-tk-Luc), and secreted alkaline phosphatase (SEAP) gene as a background. (a) RXRα, (b) RXRβ, and (c) RXRγ, based on the luciferase activity of 1 μM **1** (RXR full agonist) taken as 1.0. Circles, triangles, and squares indicate **5**, **11a**, and **11b**, respectively. The data (n = 3) represent the mean ± sem. Data for NEt-TMN were taken from ref 18, because these experiments were performed at the same time.

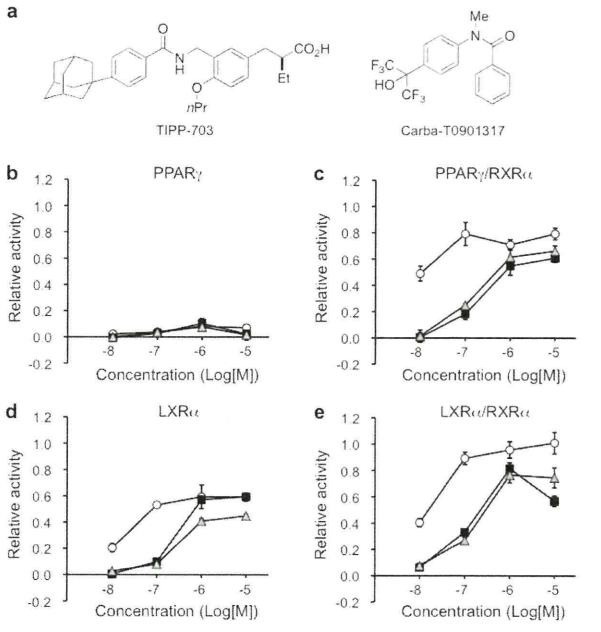


Figure 4. Relative transactivation activities of **5**, **11a**, and **11b** toward PPARγ, PPARγ/RXRα, LXRα, and LXRα/RXRα. COS-1 cells were transfected with four kinds of vectors, consisting of RXRα, a partner receptor (PPARγ or LXRα), the partner response element (tk-PPREx3-Luc for PPARγ or tk-rBARx3-Luc for LXRα), and secreted alkaline phosphatase (SEAP) gene as a background. (a) Chemical structures of TIPP703[21] (PPAR pan-agonist) and carba-T0901317[22] (LXR pan-agonist). (b) Relative transactivation data for PPARγ, based on the luciferase activity of 1 μM TIPP703 taken as 1.0. (c) Relative transactivation data for PPARγ/RXRα, based on the luciferase activity of 1 μM TIPP703 taken as 1.0. (d) Relative transactivation data for LXRα, based on the luciferase activity of 1 μM carba-T0901317 (LXR pan-agonist) taken as 1.0. (e) Relative transactivation data for LXRα/RXRα, based on the luciferase activity of 1 μM carba-T0901317 taken as 1.0. TIPP703 or carba-T0901317 at 1 μM give each E_{max} value. Circles, triangles, and squares indicate **5**, **11a**, and **11b**, respectively. The data (n = 3–6) represent the mean ± sem.

dx.doi.org/10.1021/ml300055n | ACS Med. Chem. Lett. 2012, 3, 427–432

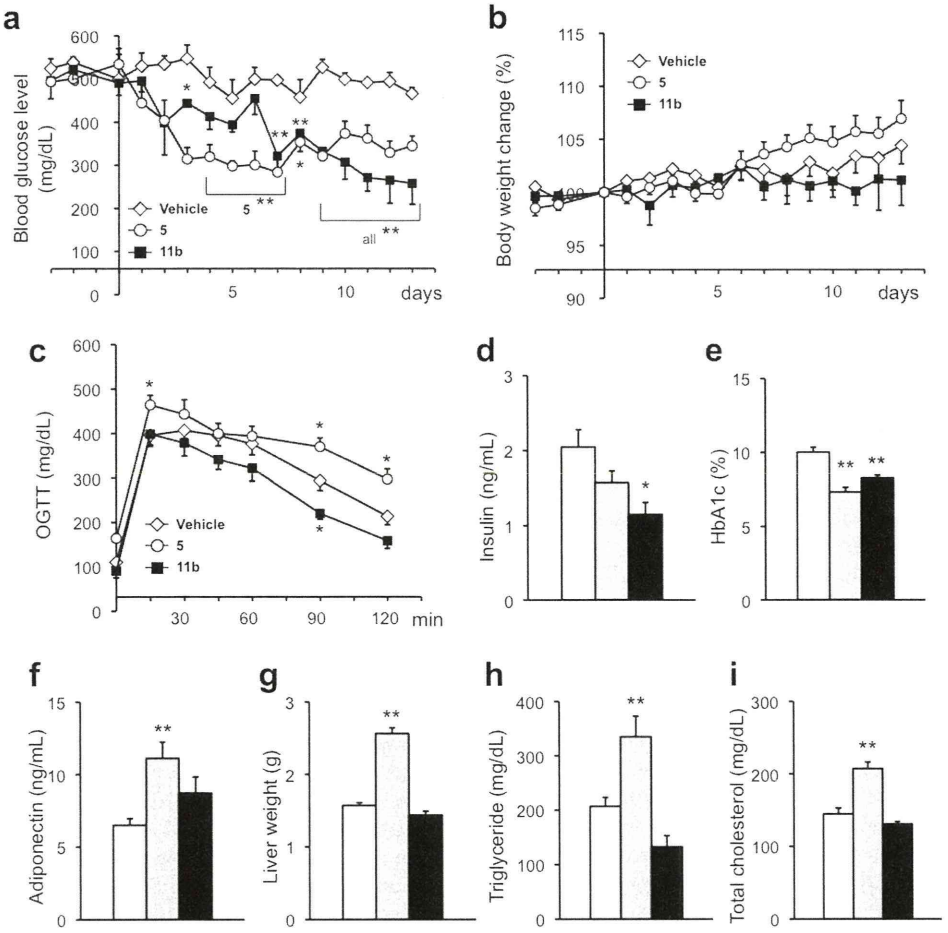


Figure 5. Evaluation of antitype 2 diabetes effects of repeated oral administration of **5** or **11b** at 10 mg/kg/day to male KK-A^{y} mice for 14 consecutive days. (a) Time course of blood glucose levels. (b) Time course of body weight change. (c) Results of oral glucose tolerance tests (OGTT) in KK-A^{y} mice treated with vehicle and compounds. (d) Effects of compounds on serum insulin levels. (e) Effects of compounds on serum HbA1c levels. (f) Effects of compounds on adiponectin levels. (g–i) Effects of compounds on liver weight, serum triglyceride, and total cholesterol, respectively. The white, gray, and black bars indicate vehicle, **5**, and **11b** treatment, respectively. The data (n = 3–7) represent the mean ± sem. Data for vehicle control and **5** were taken from ref 18, because these experiments were performed at the same time. Statistical analysis was performed by analysis of variance (ANOVA). Significant differences: * $p < 0.05$ vs vehicle. ** $p < 0.01$ vs vehicle.

obtain RXR partial agonistic activity was to form a ring between the hydrophobic or acidic domain and the linking domain to decrease the molecular flexibility.

Compounds were synthesized as illustrated in Schemes S1 and S2 (see Supporting Information) and evaluated by measuring their RXR-agonistic activities in reporter-gene assays. Although the compounds possessing ring structures between the hydrophobic and the linking domains did not show RXR agonistic activities, compounds **11a** and **11b**, in which the ring was formed between the acidic and the linking domains, did show agonistic activity (Supporting Information Table S2). Interestingly, while **11a**, possessing a 2-methylimidazole structure, showed full agonistic activity, **11b**, possessing a triazole structure, showed only partial agonistic activity toward RXRα (EC_{50} = 143 nM, E_{max} = 75%) (Figure 3a and Supporting Information Table S2). The RXR partial agonist activity of **11b** is thought to be due to not only the closed ring structure but also other factors, including the polarity/positions of the nitrogen atoms. Moreover, **11b** behaved similarly toward other RXR subtypes (Figure 3b,c). The RXR agonistic activity of the full agonist LGD1069 (**1**) at 1 μM was reduced in the presence of increasing concentrations of **11b**, indicating that **11b** acts as a partial antagonist (Supporting Information Figure S1). In addition, **11b** showed moderate RAR activation (Supporting Information Figure S2). These results support the idea that **11b** acts as a RXR partial agonist.

The activities of **11a** and **11b** toward PPARγ/RXRα and LXRα/RXRα were next examined, with RXR full agonist **5** as a positive control.[18] None of the compounds showed PPARγ activity, but compounds **11a** and **11b** showed similar PPARγ/RXRα activation, though they were both less potent than **5** (Figure 4b,c). Compounds **11a** and **11b** also activated both LXRα and LXRα/RXRα less potently than **5** (Figure 4d,e). Since these compounds showed both PPARγ/RXRα and LXRα/RXRα agonistic activities in vitro, we next examined their in vivo activities.

First, it was confirmed that oral single administration of each compound at 30 mg/kg resulted in a serum concentration over 1 μM in ICR male mice (Supporting Information Figure S3). Then, changes in body weight, hepatomegaly, and serum triglyceride (TG) elevation were assessed when each compound was administered orally to ICR mice at 30 mg/

dx.doi.org/10.1021/ml300055n | ACS Med. Chem. Lett. 2012, 3, 427–432

kg/day for 7 days (Supporting Information Figure S4). The mice given the RXR full agonists **5** and **11a** showed greater body weight gain than did the vehicle control group, and the increase was significant in the case of **5**. In contrast, the group treated with **11b** showed similar body weight change to the control group (Supporting Information Figure S4a). Liver weight was increased significantly by both **5** and **11a**, but not by **11b**, compared to the control group (Supporting Information Figure S4b). Compound **11b** also did not increase serum TG and total cholesterol values compared to the control group, whereas **5** significantly increased the TG level (Supporting Information Figure S4c,d). Further, when **11b** was administered orally to male and female SD rats at 30 mg/kg/day for 28 days, no significant difference in body weight change, water intake, or food intake was observed, compared with the vehicle control (Supporting Information Figure S5). The testes of animals treated with **11b** were slightly enlarged, but the other agents had no such effect (Supporting Information Table S4). As for serum constituents, although some significant differences from the vehicle were observed, the data were within the ranges considered normal by the suppliers (Charles River, Ltd.) (Supporting Information Table S5). Thus, it seems unlikely that the RXR partial agonist **11b** would cause the side effects associated with RXR full agonists.

Next, we examined the antitype 2 diabetes activity in male KK-A^y mice. Compound **5** or **11b** was orally administered at 10 mg/kg/day for 14 days. The average blood glucose level in vehicle-treated mice was about 500 mg/dL, while the level in **5**-treated mice was reduced to about 300 mg/dL from day 3 after the start of administration, showing a significant blood glucose-lowering effect. Compound **11b** also showed a significant blood glucose-lowering effect, although the lag time was longer than that in the case of **5** (Figure 5a). Moreover, **11b** significantly reduced serum insulin concentration (Figure 5d) and HbA1c (hemoglobin A1c, which is correlated with blood glucose levels over a period of time) (Figure 5e) and produced an improvement in the oral glucose tolerance test (OGTT) (Figure 5c), showing significant antitype 2 diabetes effects. Since **11b** improved insulin resistance in KK-A^y mice, we quantitated adiponectin, which is reported to be associated with insulin resistance, and found that **5** increased adiponectin significantly, whereas **11b** did so only moderately (Figure 5f). Since a low adiponectin level is related to insulin resistance, adiponetin elevation by RXR agonists may be correlated with their antitype 2 diabetes effects.

We also evaluated adverse effects in male KK-A^y mice treated with RXR full agonist **5** or RXR partial agonist **11b**. Examination of body weight change, liver weight, serum TG, and total cholesterol revealed that while **5** induced significant increases similar to those seen in ICR mice, **11b** did not alter these parameters in comparison with the vehicle (Figure 5b,g–i). Thus, **11b** appears to have a favorable profile of therapeutic and side effects. The reason why **11b** showed antitype 2 diabetes effects at 10 mg/kg *p.o.* but did not produce significant side effects even when orally administered at 30 mg/kg/day, which provides a serum concentration sufficient to produce the E_{max}, is considered to be its RXR partial agonist character, though other factors such as differences in timing or mechanism of action may also be involved.

To address the mechanism of the antitype 2 diabetes activity of **11b**, we examined changes in the expression levels of genes associated with glucose/lipid metabolism in the liver of KK-A^y mice by means of RT-PCR (Figure 6). It is reported that RXR

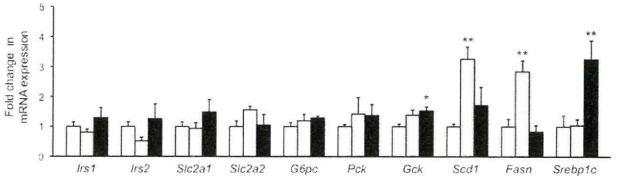


Figure 6. Fold changes in mRNA expression of *Irs1* (a), *Irs2* (b), *Slc2a1* (c), *Slc2a2* (d), *G6pc* (e), *Pck* (f), *Gck* (g), *Scd1* (h), *Fasn* (i), and *Srebp1c* (j) in the liver tissue of male KK-A^y mice treated with vehicle, **5**, or **11b** at 10 mg/kg/day for 14 consecutive days. These measurements were performed using the same mice as in the case of Figure 5. The white, gray, and black bars indicate vehicle, **5**, and **11b** treatment, respectively. The data (n = 3–7) represent the mean ± sem. Statistical analysis was performed by analysis of variance (ANOVA). Significant differences: * $p < 0.05$ vs vehicle. ** $p < 0.01$ vs vehicle.

agonists increase *Slc2a1* (GLUT1) and *Slc2a2* (GLUT2), thereby inducing an increase of liver glucose intake.[23] *Gck* expression is also increased by RXR agonists.[23] Other changes include suppression of *G6p* and *Pck* expression, and increase in expression of *Gck* (related to glycolysis), *Scd1*, *Fasn*, and *Srebp1c*, which are associated with increased lipid synthesis induced by LXR agonists.[6] We also examined changes in expression of *Irs1* and *Irs2*. Neither **5** nor **11b** influenced gene expression of *Irs1*, *Irs2*, *Slc2a1*, *Slc2a2*, *G6pc*, and *Pck*. However, **11b** increased *Gck* expression significantly, indicating that one of the mechanisms of the glucose-lowering effect of **11b** is induction of glycolysis via *Gck*. While **5** markedly increased the gene expression of *Scd1* and *Fasn*, **11b** did not. Since *Scd1* and *Fasn* are associated with lipid synthesis, this result may explain the lack of serum TG elevation by **11b**.

Expression of *Gck*, *Scd1*, and *Fasn* is regulated by *Srebp1c*,[24−26] but **5** had no effect on the expression of *Srebp1c*. It has been reported that *Sreb1c* expression in liver is suppressed by adiponectin.[27] Therefore, the reason why **5** had no effect on *Srebp1c* expression may be that it induced a significant increase of adiponectin, as shown in Figure 6. On the other hand, the partial agonist **11b** activates RXR only moderately, and this may be sufficient to induce expression of *Srebp1c*, which lowers blood glucose, without causing overexpression of *Scd1* and *Fasn*, which induce lipid synthesis. These results are consistent with our hypothesis that there is a threshold difference between the therapeutic and adverse effects of RXR activation. Although an appropriate low dosage of a RXR full agonist may show similar beneficial effects to a RXR partial agonist **11b**, in the case of the overdose medical malpractice, RXR full agonists can cause several adverse effects. Therefore, RXR partial agonists will be more attractive antitype 2 diabetes drug candidates than RXR full agonists.

In summary, we hypothesized that there are different thresholds for the therapeutic effects and side effects of RXR activation. Therefore, we aimed to synthesize RXR partial agonists by reducing the molecular flexibility of the representative RXR agonist structure. As we had hoped, ring formation between the acidic and linking domains with a triazole structure afforded a RXR partial agonist **11b**. This compound showed a significant antitype 2 diabetes effect in KK-A^y diabetic model mice but did not induce the side effects associated with RXR full agonists in ICR mice or SD rats. Compound **11b** did not induce expression of genes associated with lipid synthesis, whereas the full agonist **5** did induce expression of these genes. These results support our hypothesis

dx.doi.org/10.1021/ml300055n | ACS Med. Chem. Lett. 2012, 3, 427–432