投与群において，day 5 には体重の改善傾向を示し，腸長についても改善が見られた．NEt-4IB (**15a**) 投与群は腸の炎症所見の改善が見られ，肝肥大は認められなかった．

さらに著者らは，NEt-3IB と NEt-4IB (**15a**) の安全係数 (Safty Index : SI) を算出できないかと考えた．SI は 50%有効量を表す ED_{50} と，50%致死量を表す LD_{50} の比 (LD_{50}/ED_{50}) で表され，値が大きいものほど安全性の高い薬物であることを示すが，実際にこれを行おうとすると多くの動物や手間が必要となる．そこで，SI を薬効/副作用という形で表そうと考えた．即ち著者は，SI = 腸長/ [(肝重量/体重) × 100] と定義し，SI の算出を行った (Figure 20)．この値は薬効と副作用のバランスを示したものであるが，大きいものほど副作用が少ない，もしくは薬効が強い，あるいはその両方であることを表している．その結果，vehicle 群と比べて，NEt-3IB 投与群では腸長が改善しておらず，副作用

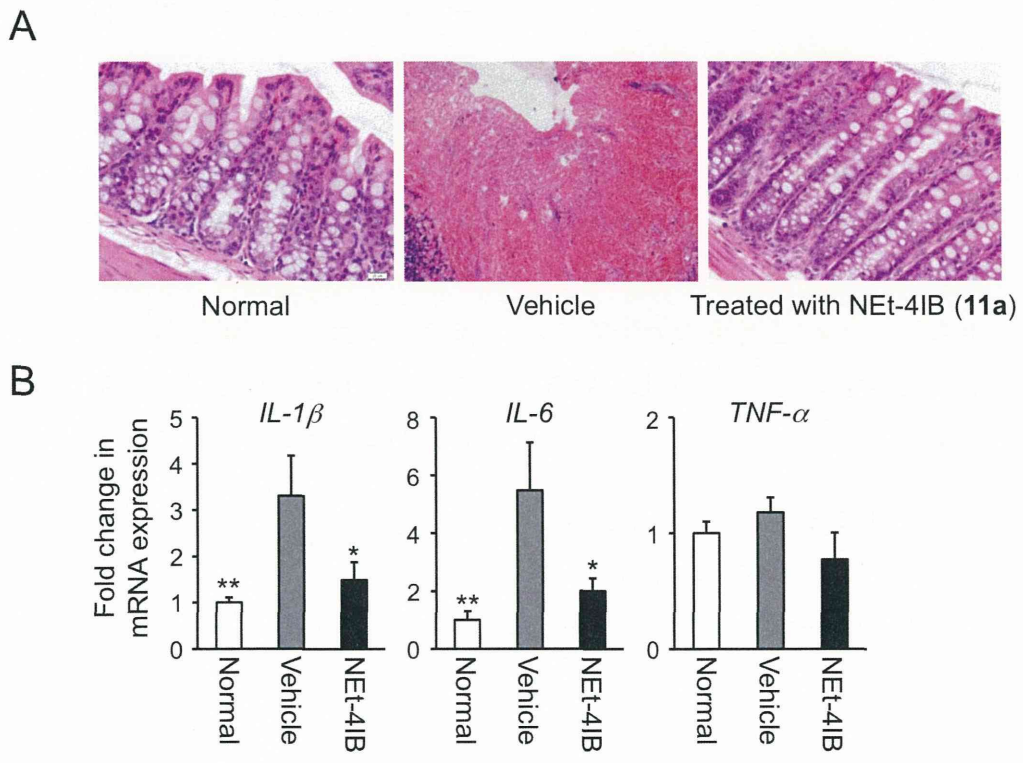

Figure 21. Anti-inflammatory effect of NEt-4IB (**15a**) in NBD-Cl induced inflammatory bowel disease model mice. A) Representative histological sections of colon tissues of Balb/c mice. B) Fold changes in mRNA expression of IL-1β, IL-6, and TNF-α in the colon tissue of normal mice or NBD-Cl induced bowel disease model mice treated with vehicle and NEt-4IB (**15a**) at 0.2 mg × 2/day for 4 consecutive days. 5. The white, gray, and black bars indicate normal, vehicle, and NEt-4IB (**15a**) treatment, respectively. Data shown are the average (n = 6-9) ± SEM and analyzed by one-way ANOVA followed by Bonferroni test. * : $p < 0.05$ and ** : $p < 0.01$ vs. vehicle respectively.

として肝肥大が起きているため，SI 値の低下が見られるが，NEt-4IB (**15a**) 投与群では腸長の改善が見られる一方で，肝肥大が起きていないため，SI 値は上昇している．

解剖時に大腸を採取し，ホルマリンで固定し，切片の作成した．その結果をFigure 21に示す．炎症誘発群であるvehicleは明らかに組織形態がnomarl群に比べ破壊され，顕著な炎症状態が確認された．一方で，炎症誘発群にNEt-4IBを投与した群においては，normal群に匹敵する改善が見られた．

炎症性サイトカインとして知られるIL-1β，IL-6，TNF-α の測定を行ったところ，炎症誘発群である vehicle においてIL-1β, IL-6 の明らかな上昇が確認された．一方で NEt-4IB (**15a**) 投与群の大腸は，normal 群に匹敵するまでに回復していることが確認できた．

NEt-3IB 投与において腸炎の改善が見られなかった理由については，詳細なことは分かっていないため，今後の検討課題である．なお，本モデルは TNBS 誘発クローン病モデルマウスより安定してモデル作成ができ，再現性も確認されている．

【参考文献】

22. *J. Exp. Med.*, 2001, *193*, 827–838.

１３．Imiquimod 誘発乾癬モデルでの薬効評価

イミキモド（Imiquimod：ベセルナクリーム® 持田製薬株式会社）は，樹状細胞やマクロファージなどに発現している Toll-like receptor 7（TLR-7）に直接結合し活性化することが知られ，マウスの皮膚に反復塗布することで乾癬モデルマウスが作成出来ることが報告されている.[18] 本モデルでは Th17 細胞の顕著な増加が見られる．Th17 細胞発現の抑制において Treg 誘導が有効であることが報告されていることから,[23] 本モデルにおける創出化合物の有効性が期待された．

Figure 22 に，乾癬モデルマウスの体重変化ならびに病変部位の写真を掲載する．CBt-PMN ならびに NEt-4IB (**15a**)とも，10 mg/kg/day の反復投与により炎症の改善が見られ，とくに NEt-4IB (**15a**)の効果が顕著であった．

本モデルにおいて，炎症が誘発されると顕著な脾臓肥大化，さらに脾臓細胞中の $CD4^+$細胞において Th17 細胞（IL-17 発現 $CD4^+$細胞）の誘導が認められる．[18] この状態に対し，NEt-4IB (**15a**)の投与により脾臓肥大化の抑制，また Th17 細胞の誘導が抑制されていれば，投与薬物の薬効が示されると考えた．また，制御性細胞（Treg: $Foxp3^+$発現 $CD4^+$細胞）の誘導が確認されるかについても，興味が持たれた．そこで，岡山大学大学院医歯薬学総合研究科の谷本光音教授，前田嘉信講師，西森久和助教に協力を得て，本実験で得られた脾臓細胞について，その細胞組成についてフローサイトメーターにより解析して頂いた．その結果を Figure 23 に示す．Imiquimod 誘発炎症群（vehicle）において，normal 群に対して顕著な脾臓細胞数の増

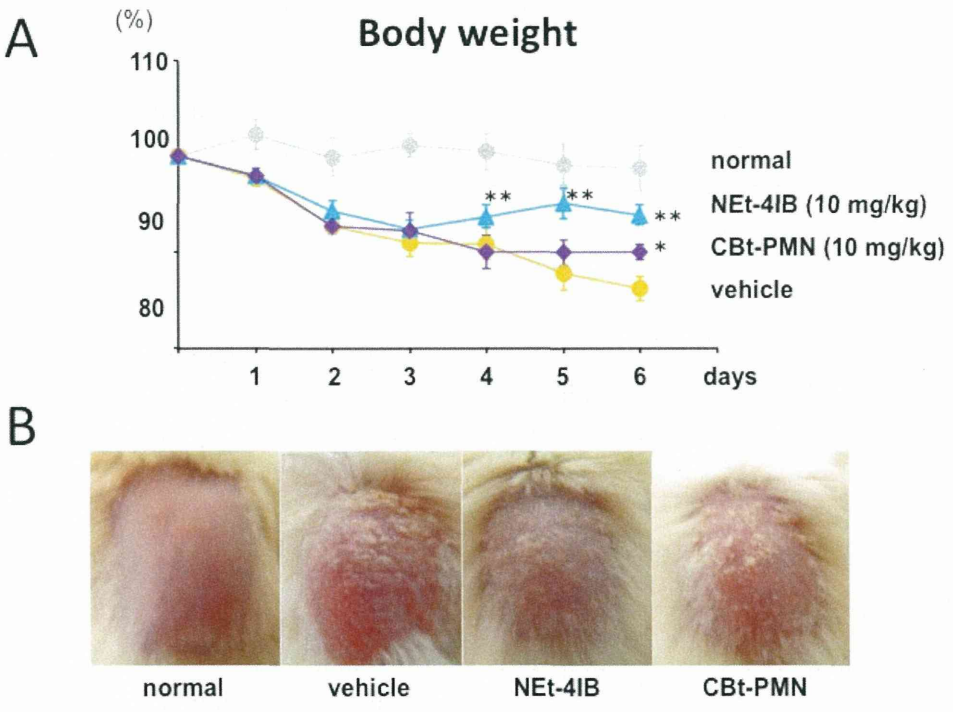

Figure 22. Imiquimod-induced skin inflammation in mice phenotypically resembles psoriasis. A) Body weight changes. B) Phenotypical presentation of mouse back skin after 6 days of treat- ment. Data shown are the average (n = 6) ± SEM and analyzed by one-way ANOVA followed by Bonferroni test. * : $p < 0.05$ and ** : $p < 0.01$ vs. vehicle respectively.

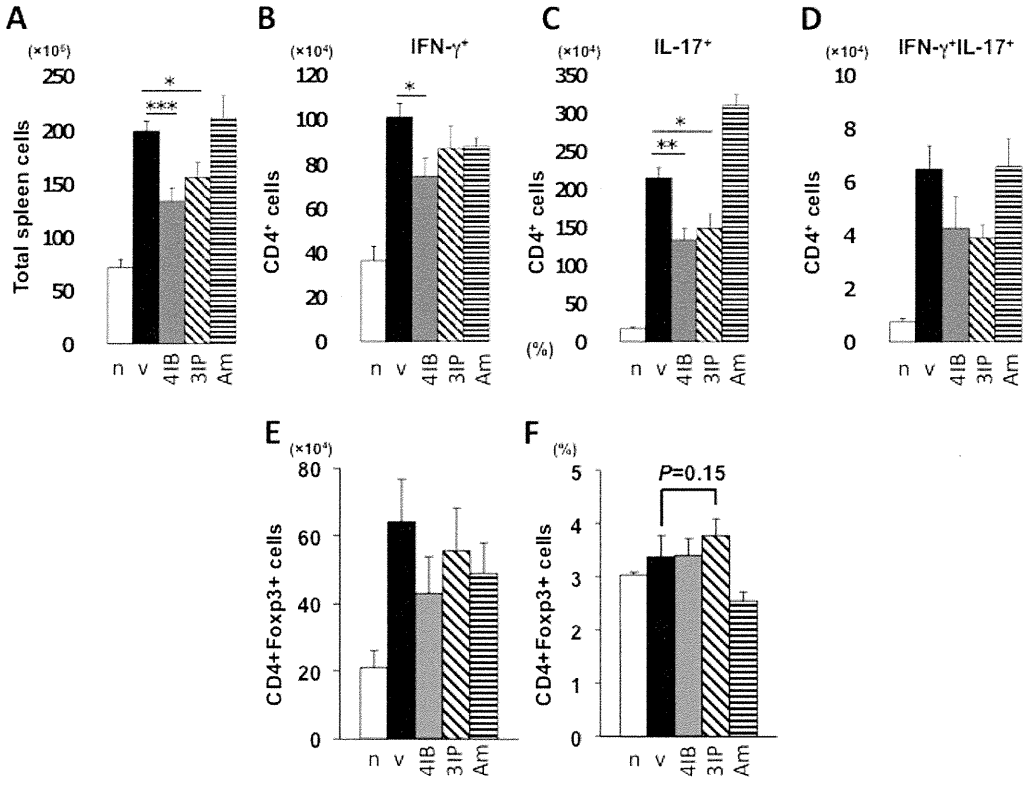

Figure 23. Topical imiquimod (IMQ) increases spleen mass and alters its cellular composition. Mice were treated with IMQ or control cream for 6 consecutive days. A, Mice were sacrificed and spleen total cells were determined. B–F, Spleen cells were analyzed for the cell numbers or percentage of T cells by flow cytometry. Data shown are the average (n = 5) ± SD and analyzed by t-test. * : $p < 0.05$, ** : $p < 0.01$ and *** : $p < 0.005$ vs. vehicle respectively.

加，さらに Th1 細胞（IFN-γ発現 $CD4^+$細胞），Th17 細胞（IL-17 発現 $CD4^+$細胞），Th1/17 細胞（IFN-γ・IL-17 発現 $CD4^+$細胞）が有意に上昇していたのに対して，Treg 誘導能が確認されている RXR フルアゴニスト NEt-3IP および RXR パーシャルアゴニスト NEt-4IB 投与により，これらの発現が抑制もしくは抑制傾向を示した．一方で，Treg 誘導能かつ Th17 の発現抑制が報告されている RARα/βフルアゴニストである Am80[24] では，vehicle 群と変わらないことが分かった．

Treg 細胞（$Foxp3^+$発現 $CD4^+$細胞））については，NEt-3IP 投与群で上昇傾向が見られたが（Figure 12F），顕著なものではなかった．また，Am80 投与群における Treg 細胞は，vehicle 群に比べ低下した．

以上から，NEt-3IP および NEt-4IB による Th1 細胞（IFN-γ発現 $CD4^+$細胞），Th17 細胞（IL-17 発現 $CD4^+$細胞），Th1/17 細胞（IFN-γ/IL-17 発現 $CD4^+$細胞）の発現抑制作用は，Treg 誘導を介したものかは不明であり，今後の検討が必要である．

なお，組織切片，PCR 等による炎症性サイトカインの発現解析等を行うに至っていないため，引き続き本モデルでの有効性を調べるべく，継続して実験を行う予定である．

【参考文献】

23. *Pediatric Research*, 2009, *65*, 26R–31R.
24. *Am. J. Pathol.* 2009, *174*, 2234–2245.

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．

平成24年度にはCBTF-PMN (**1d**)について，ラットへの28日間連続投与を行った．その結果，フルアゴニストに比べて明らかに弱いものの，わずかな肝肥大が見られた．しかし，これまでのRXRフルアゴニストに見られた血中トリグリセリドの上昇は回避出来ており，なおかつ本化合物がTNBSモデルでも薬効を示したことから，RXRパーシャルアゴニストの有用性に期待がもたれた．

RXR パーシャルアゴニストの in vivo における報告は著者らの見出した CBt-PMN および CBTF-PMN に限られ，さらにこれらの化合物の分子構造は互いに類似する．そこで，RXR パーシャルアゴニストであれば副作用を回避しつつ薬効を示すことの一般性を調べること，さらに本研究の目的とするクローン病や乾癬などの免疫疾患治療に有効な新たな小分子型医薬候補物質たる RXR パーシャルアゴニストの創出を目指し，新たな RXR パーシャルアゴニストの創出，その薬効・副作用発現について調べた．

合成した化合物の中でも疎水性部位 4’

位にイソブトキシ基を有する化合物NEt-4IB (**11a**)に最もEC_{50}の小さいRXRパーシャルアゴニスト活性 (E_{max} = 55%, EC_{50} = 169 nM) を見出した．さらに，NEt-4IB (**11a**)を 30 mg/kg で経口投与し，血中移行性を調べたところ，良好な血中移行性 (C_{max} = 13.2 μM) を示すことが分かった．

このように新規RXRパーシャルアゴニストNEt-4IB (**11a**)を見出したことから，本化合物の副作用発現を調べた結果，マウスへの1週間連続投与，ラットへの28日間連続投与いずれにおいてもRXRフルアゴニストに見られるような体重増加，肝肥大，および血中TG値の上昇などの副作用は見られなかった．また，4-chloro-7-nitro-2,1,3-benzoxadiazole (NBD-Cl) 溶液を腸注することでクローン病モデルマウスを作成し，化合物の投与を行うことで薬効である抗炎症作用の評価を行ったところ，NEt-4IBの投与によって体重や腸長の改善が見られた．大腸のPCRを行い，炎症性サイトカインの発現を調べた結果，IL-1βやIL-6の顕著な低減が見られ，NEt-4IBの抗炎症作用が示された．さらに，imiquimodによる乾癬モデルを作成し，これにおいても有効なことを確認した．なおこれらの成果は，新たな特許出願に至っている（特願2012-224474）．

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．

本研究により，研究当初に取り扱っていたRXRパーシャルアゴニストCBt-PMNより顕著な薬効を示しながら，RXRフルアゴニストに見られる副作用発現を回避した新規RXRパーシャルアゴニストNEt-4IBの発見に至った．さらに，NEt-4IBはCBt-PMNと分子構造が明らかに異なりながら，クローン病モデルマウスでの有効性が示されたことから，RXRパーシャルアゴニストであれば一般的に副作用を回避しつつ薬効を示すことが示唆された．さらに，乾癬モデルマウスでの有効性も示唆された．

Imiquimod乾癬モデルマウスにおいてTh17の発現抑制が見られることから，本事業において計画していたリウマチモデルでの薬効も期待した．東京医科歯科大学の上阪准教授に，コラーゲン誘発マウス関節炎モデルでNEt-4IBの薬効を評価していただいたものの，顕著な効果は見られなかった．

これまでRXRアゴニストは脂質代謝異常症やアルツハイマー病等にも有効性が報告されているが，その副作用発現が問題となっており，臨床応用はされていなかった．しかし，RXRパーシャルアゴニストであれば副作用が回避できることが示唆されたため，本研究を通じて見出されたNEt-4IB，もしくはその他のRXRパーシャルアゴニストを今後精査することで，本事業で対象とした自己免疫疾患に限らず，これまでにRXRフルアゴニストで報告のある糖尿病，脂質異常症，およびアルツハイマー病などに対する新薬候補としても期待がもたれる．

本事業に関連させて，CBt-PMNおよびNEt-4IBについてOECD準拠によるAmes試験を岡山大学大学院医歯薬学総合研究科 有元佐賀恵准教授により行って頂いた．その結果，対象とした5菌株に対しいずれも陰性であり，変異原性は認められなかった．

なお，プロジェクトとしての最終目標は，創出化合物による免疫疾患罹患者への医療貢献であるが，申請者らのグループのみで達成できるものではなく，製薬企業等との共同研究が必須である．そのようなことから，本申請研究の最終目標として，前臨床/臨床試験を行う企業等との共同研究化につなげることを目指し，①構造の異なるRXRパーシャルアゴニスト10種以上，②病体モデルで有効な化合物3種以上，③特許出願2報以上，④論文4報以上，という数値目標を掲げ実施した．①から③については数値目標を達し，④について，現時点での掲載論文数は2報であるものの，新規RXR パーシャルアゴニスト NEt-4IB については投稿準備中であること，また各化合物の病態モデルでの薬効についても未発表であることから，これらについて論文投稿予定である．

また，これらの成果をもとにBioJapan2012 などでの広報を行い，多数の企業からの問い合わせをいただき，現在，共同研究について協議中である．

F. 研究発表

1．論文発表

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Tai A, Yoshikawa Y, Yasui H, Kakuta H*. Mechanism of Retinoid X Receptor Partial Agonistic Action of 1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-1*H*-benzotriazole-5-carboxylic Acid and Structural Development To Increase Potency. *J. Med. Chem.*, 2013, *56*, 1865−77.

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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

名称: レチノイドX受容体パーシャルアゴニスト化合物及びこのレチノイドX受容体パーシャルアゴニスト化合物を有効成分として含有する薬剤，特願2012-224474，出願日：平成24年10月9日，発明者：加来田博貴，出願人：岡山大学

II. 研究成果の刊行に関する一覧表

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

Fuminori Ohsawa, Shoya Yamada, Nobumasa Yakushiji, Ryosuke Shinozaki, Mariko Nakayama, Kohei Kawata, Manabu Hagaya, Toshiki Kobayashi, Kazutaka Kohara, Yuuki Furusawa, Chisa Fujiwara, Yui Ohta, Makoto Makishima, Hirotaka Naitou , Akihiro Tai , Yutaka Yoshikawa, Hiroyuki Yasui, and Hiroki Kakuta	[b]Mechanism of Retinoid X Receptor Partial Agonistic Action of 1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-1H-benzotriazole-5-carboxylic Acid and Structural Development To Increase Potency	Journal of Medicinal Chemistry	56	1865–1877	2013

a) 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．

b) 本事業にて行った RXR パーシャルアゴニスト CBTF-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**，**Figure S7**，**Table S1**，**Table S2** および **Table S4** である．

III. 研究成果を含む刊行物

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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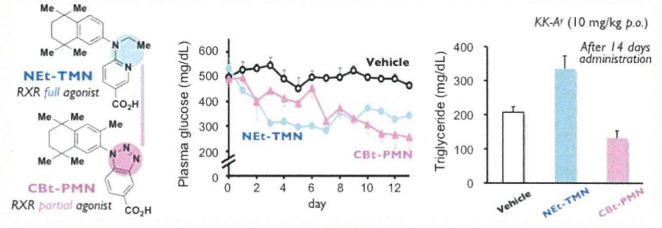
Supporting Information

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-Aʸ 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

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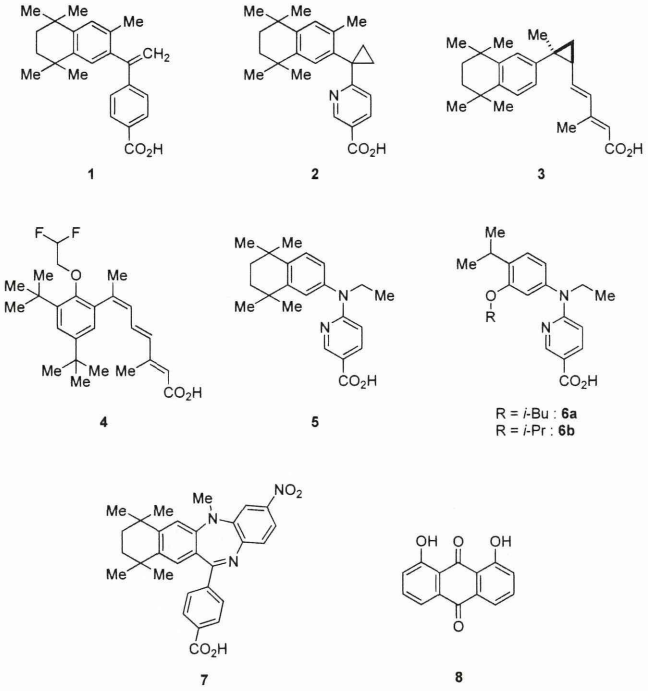

Figure 1. Chemical structures of known RXR ligands.

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

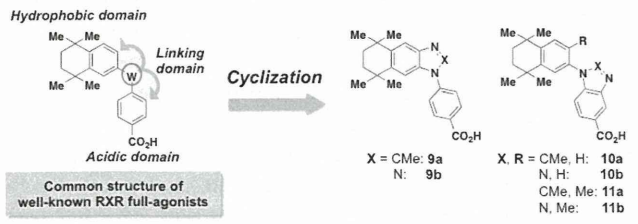

Figure 2. Molecular design strategy for creating RXR partial agonists.

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

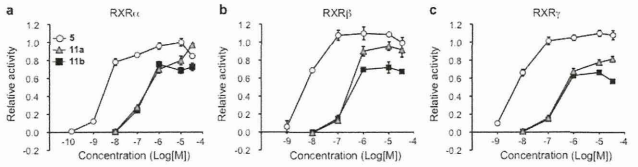

Figure 3. Results of reporter gene assays of **5**, **11a**, and **11b**. COS-1 cells were transfected with three kinds of vectors consisting of a RXR receptor subtype, a luciferase reporter gene under the control of the appropriate RXR response element (CRBPII-tk-Luc), and secreted alkaline phosphatase (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.

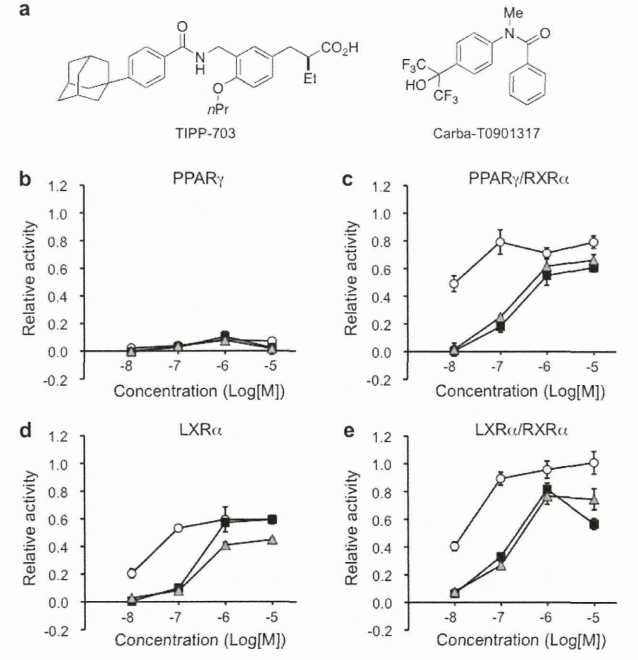

Figure 4. Relative transactivation activities of **5**, **11a**, and **11b** toward PPARγ, PPARγ/RXRα, LXRα, and LXRα/RXRα. COS-1 cells were transfected with four kinds of vectors, consisting of RXRα, a partner receptor (PPARγ or LXRα), the partner response element (tk-PPREx3-Luc for PPARγ or tk-rBARx3-Luc 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.

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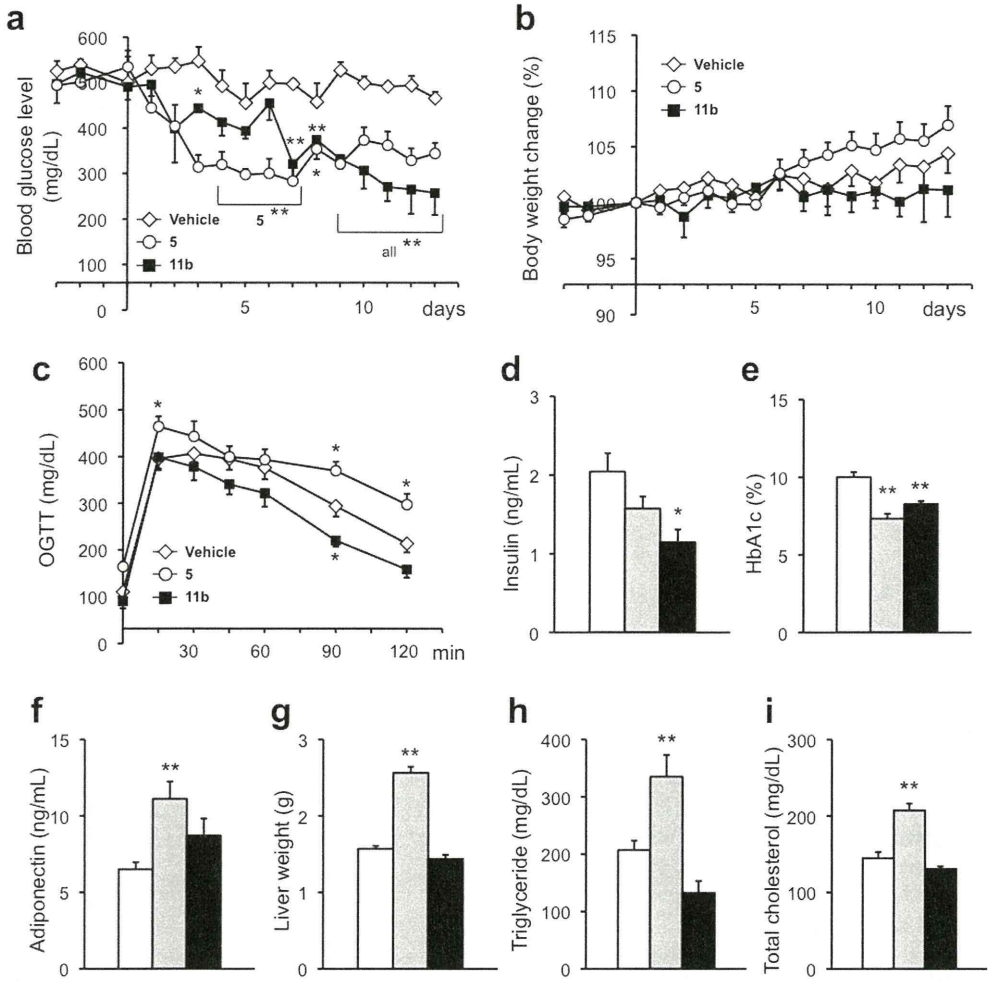

Figure 5. Evaluation of antitype 2 diabetes effects of repeated oral administration of **5** or **11b** at 10 mg/kg/day to male KK-A^y mice for 14 consecutive days. (a) Time course of blood glucose levels. (b) Time course of body weight change. (c) Results of oral glucose tolerance tests (OGTT) in KK-A^y 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/

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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

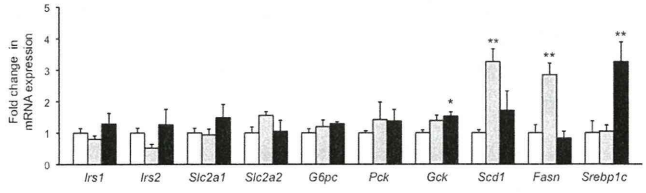

Figure 6. Fold changes in mRNA expression of *Irs1* (a), *Irs2* (b), *Slc2a1* (c), *Slc2a2* (d), *G6pc* (e), *Pck* (f), *Gck* (g), *Scd1* (h), *Fasn* (i), and *Srebp1c* (j) in the liver tissue of male KK-A^y mice treated with vehicle, **5**, or **11b** at 10 mg/kg/day for 14 consecutive days. These measurements 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

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of a threshold difference for the therapeutic and side effects of RXR agonists. We believe that RXR partial agonists such as **11b** represent a promising class of candidate antitype 2 diabetes agents.

■ ASSOCIATED CONTENT

Supporting Information

General information, synthetic procedures, combustion analysis data, HPLC charts, luciferase reporter gene assay, and in vivo experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

H.K. conceived and designed the project. N.Y., R.S., and M.H. synthesized compounds. F.O., S.Y., and Y.O. performed reporter gene assays. M.M. prepared plasmids. K.K., M.N., and C.F. performed in vivo experiments with ICR mice and SD rats. A.T. performed HPLC analysis. Y.Y. and H.Y. performed in vivo experiments with KK-A^y mice. C.F., S.U., A.M., M.N., and T.O. performed PCR analysis. The manuscript was written by H.K. and F.O.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

RXR, retinoid X receptor; PPAR, peroxisome proliferator-activated receptors; LXR, liver X receptor; RAR, retinoic acid receptor; TG, triglyceride; RT-PCR, reverse transcriptase polymerase chain reaction; *Irs*, insulin receptor substrate; GLUT, glucose transporter; *G6p*, glucose-6-phosphatase; *Pck*, phosphoenolpyruvate carboxykinase; *Gck*, glucokinase; *Scd1*, stearoyl-CoA desaturase 1; *Fasn*, fatty acid synthase; *Srebp1c*, sterol regulatory element-binding protein 1c

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Supporting Information

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

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