Synthesis of 2-Prenylated Alkoxylated Benzopyrans by Horner-Wadsworth-Emmons olefination with PPARα/γ Agonist Activity

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ABSTRACT: We have synthesized series of 2-prenylated benzopyrans as analogs of the natural polycerasoidol, a dual PPARα/γ agonist with anti-inflammatory effect. The prenylated side chain consists of five- or nine-carbons with an α-alkoxy-α,β-unsaturated ester moiety. Prenylation was introduced via Grignard reaction, followed by Johnson-Claisen rearrangement, and the α-alkoxy-α,β-unsaturated ester moiety by the Horner-Wadsworth-Emmons reaction. Synthetic derivatives showed high efficacy to activate both hPPARα and hPPARγ as dual PPARα/γ agonists. These prenylated benzopyrans emerge as lead compounds potentially useful for preventing cardiometabolic diseases.

KEYWORDS: *Prenylated benzopyrans, Horner-Wadsworth-Emmons reaction, PPARα/γ activity*

Among nuclear receptor family, peroxisome proliferator activated receptors (PPARs) are transcription factors activated by ligands, which are implicated in numerous cell functions including glucose and lipid metabolism, and inflammation.[1] Dual PPARα/γ activators can improve atherogenic dyslipidemia and insulin resistance.[1] Polycerasoidol is a *trans*-δ-tocotrienolic acid analog[2] isolated from *Polyalthia cerasoides* (Annonaceae).[3,4] Polycerasoidol, containing a 6-chromanol nucleus and a 2-prenylated side chain, is biosynthesized from homogentisate and geranylgeranyl pyrophosphate via the shikimate and the mevalonate pathway, respectively. Pharmacologically, it displays dual PPARα/γ agonist activity and ameliorates inflammation of dysfunctional endothelium.[5] In structural terms, this natural benzopyran possesses a benzopyran nucleus (lipophilic tail), a prenylated chain (flexible linker), and a carboxylic acid (polar head), as other natural and synthetic PPARα and/or PPARγ agonists.[6] The structure-activity relationships (SAR) of polycerasoidol and semisynthetic analogs revealed that the 6-oxygenated dihydrobenzopyran core and the linker hydrocarbon chain of at least a five-carbon length are essential moieties to activate PPARα and/or PPARγ.[7] It is noteworthy that chroman-6-ol pharmacophore is present in troglitazone, the first PPARγ agonist belonging to the class of thiazolidinediones, which was approved as anti-diabetic agent.[8,9] Currently, rosiglitazone and pioglitazone are selective PPARγ agonists used to manage hyperglycemia in type 2 diabetes, while saroglitazar and lobeglitazone are PPARα/γ activators (agonists) approved against diabetes in India and South Korea, respectively.[9] In order to find new active and safer PPAR activators, we describe the synthesis and PPAR activity of 2-prenylated benzopyrans that bear an α-alkyloxy-α,β-unsaturated ester as a tri-oxygenated polar head on the prenylated side chain.

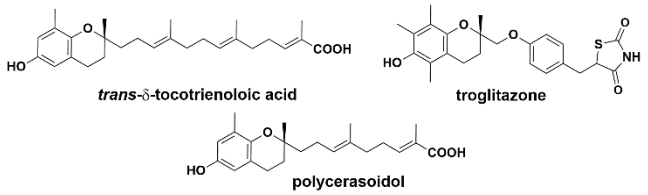
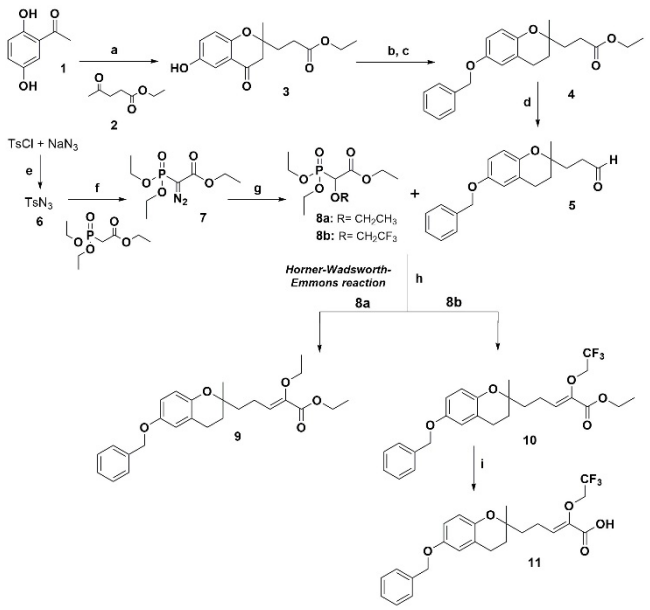


Figure 1. Bioactive prenylated benzopyrans.

We first prepared the benzopyran-4-one nucleus (γ-benzopyrone **3**) by conventional methods.[10] The reaction mechanism consists of an aldol condensation between the enolate from an *ortho*-hydroxyacetophenone and the carbonyl group from an alkyl ketone. This is followed by an intramolecular cyclisation via Michael addition, which is promoted by the *ortho*-phenol group.[11] Thus, 2,5-dihydroxyacetophenone (**1**) and ethyl levulinate (**2**) in the presence of pyrrolidine gave the chroman-4-one **3** as a racemic mixture in a single step with a good yield (80 %) (Figure 2).[7,10] γ-Benzopyrone 3 was reduced under Clemmensen conditions and the phenol hydroxyl group at 6-position was protected by *O*-benzylation to give compound **4** with a good overall yield. The controlled reduction of the ester function by DIBAL-H at -78°C gave the aldehyde **5** with a 92 % yield. In a first approach, we synthesized five-carbon side chain alkoxylated prenylated benzopyrans (series 1) from aldehyde intermediate **5** by Horner-Wadsworth-Emmons olefination. For this purpose, appropriate

alkylphosphonates, e.g., ethyl 2-(diethoxyphosphoryl)-2-ethoxyacetate (**8a**) and ethyl 2-(diethoxyphosphoryl)-2-(2,2,2-trifluoroethoxy)acetate (**8b**), were previously prepared from (ethoxyacetate) diethoxyphosphorane and TsN_3 (**6**) in the presence of NaH.[12] Then, aldehyde intermediate **5** was reacted with phosphonate **8a** or **8b** to afford the α-alkyloxy-α,β-unsaturated esters of prenylated benzopyrans **9** (20 %, *Z/E*= 60:40) or **10** (84 %, *Z/E*= 35:65), respectively. The saponification of ethyl ester (**10**) quantitatively afforded carboxylic acid (**11**, *Z/E*= 40:60) (Scheme 1).

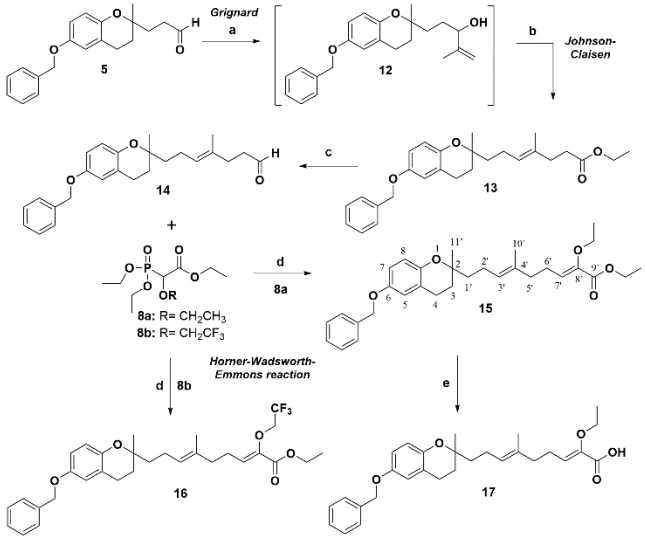
Scheme 1. Synthesis of prenylated benzopyrans **9-11** (series 1).[a]



[a] Reaction conditions: (a) Pyrrolidine, EtOH, 60 ºC, molecular sieve 3Å, 24h, 80 %; (b) Zn/HCl, AcOH-H_2O, rt, 2h; (c) $ClCH_2C_6H_5$, K_2CO_3, EtOH, 60 ºC, 5h, 90 %; (d) DIBAL-H, CH_2Cl_2, -78 ºC, N_2, 20 min, 92 %; (e) 0 ºC, acetone, 2h, 81 %; (f) 60 % NaH, THF, 0 ºC, N_2, 16h, 65 %; (g) EtOH, $Rh(OAc)_2$, toluene, 45 ºC, overnight, **8a** (R= CH_2CH_3), 40.2 % or **8b** (R= CH_2CF_3), 51 %; (h) Phosphonate **8a** or **8b**, THF, NaH, 0 ºC, N_2, 1h + **5**, THF, rt, overnight: **9**, 20 % or **10**, 84 %.; (i) 20 % KOH, reflux, 5h, 99 %.

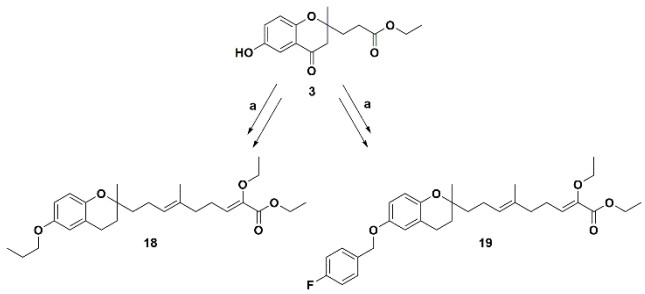
In a second approach, we synthesized the nine-carbon side chain *O*-alkoxylated prenylated benzopyrans (series 2) **15**, **16** and **17** from aldehyde intermediate **5**. The prenylated side chain at the 2-position of the dihydrobenzopyran nucleus was elongated with a sequence of Grignard reaction, Johnson-Claisen rearrangement and Horner-Wadsworth-Emmons olefination (Scheme 2). The aldehyde synthon **5** was treated with isoprenylmagnesium bromide as the vinyl Grignard reagent, followed by Johnson-Claisen rearrangement of allylic alcohol **12** using ethyl orthoacetate to produce unsaturated ester **13** with a 50 % yield in the last two steps. Ester **13** was subjected to a controlled reduction using DIBAL-H at -78°C to give the aldehyde intermediate **14** in 89 % yield. The α-alkoxy-α,β-unsaturated ester on the prenylated side chain was introduced by a Horner-Wadsworth-Emmons reaction.[13] Thus, aldehyde **14** was treated with phosphonates **8a** and **8b** to afford esters **15** (85 %) and **16** (82 %), respectively.[14] It is noteworthy that ester **15** was obtained as *Z*-alkene isomer exclusively. Once again, the saponification of ethyl ester **15** quantitatively yielded carboxylic acid **17** (Scheme 2). In addition to *O*-benzyloxy benzopyrans bearing a nine-carbon prenylated alkoxy side chain (series 2), we accomplished the synthesis of its *O*-propyloxy and *O*-*p*-fluorobenzyloxy benzopyran analogs. According to the second approach followed to prepare ester **15**, but starting from the chroman-4-one **3**, we synthesized *O*-propyloxy ester **18** and *O*-*p*-fluorobenzyloxy ester **19** (Scheme 3).

Scheme 2. Synthesis of prenylated benzopyrans **15-17** (series 2).[a]



[a] Reaction conditions: (a) $CH_3C(MgBr)=CH_2$, THF, -78 ºC, N_2, 1h, 84 %; (b) $MeC(OEt)_3$, isobutyric acid, 140 ºC, 2h, 50 %; (c) DIBAL-H, CH_2Cl_2, -78 ºC, N_2, 20 min, 89 %; (d) Phosphonate **8a** or **8b**, THF, NaH, 0 ºC, N_2, 1h + **14** in THF, rt, overnight: **15** (R= CH_2CH_3), 85 % or **16** (R= CH_2CF_3), 82 %; (e) 20 % KOH, reflux, 5h, 99 %.

Scheme 3. Synthesis of prenylated benzopyrans **18** and **19**.[a]



[a] Reaction conditions: (a) See reagents and conditions described in Scheme 1 for synthesis of **5**, and Scheme 2 for synthesis of **15** and **16**.

The transactivation studies[15] of the synthesized benzopyrans were carried out and compared to the maximal efficacy of WY-14,643 (at 10 μM) or rosiglitazone (at 1 μM) as hPPARα and hPPARγ reference compounds, respectively. At

the 10 μM dose, compounds **10**, **15**, **16** and **18** were moderate hPPAR activators for both receptors, while compounds **11**, **17** and **19** showed high efficacy as dual hPPARα/γ agonists. Indeed, compound **11** showed higher efficacy to activate hPPARα than PPARγ (α/γ ratio= 1.73), and **17** displayed slight selectivity towards hPPARγ (α/γ ratio= 0.64). Therefore, the elongation of the side chain from five- to nine-carbons is beneficial to activate hPPARγ. In agreement with previous docking analysis of polycerasoidol, the carboxylic moiety at the C-9′ position of **17** plays a key role as an anchoring point to bind PPARγ receptor.[5]

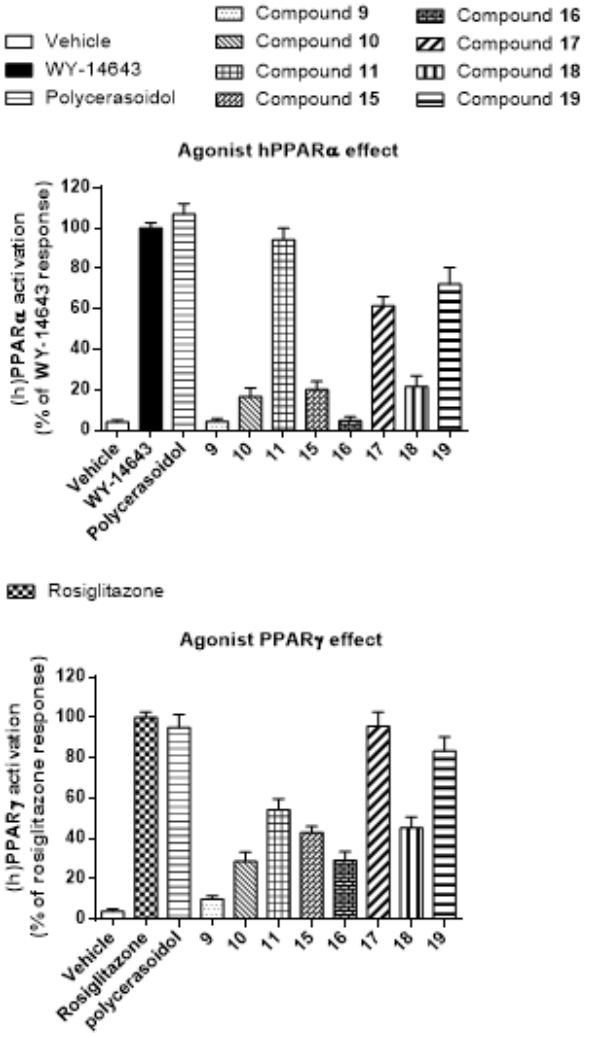


Figure 2. hPPARα and hPPARγ transactivation assays. Synthesized benzopyrans were tested at 10 μM, and WY-14,643 (10 μM) and rosiglitazone (1 μM), as reference compounds for α and γ, respectively.

In conclusion, we efficiently prepared new series of the 2-prenylated *O*-alkoxylated benzopyrans possessing the α-alkoxy-α,β-unsaturated moiety on the prenylated chain by the Horner-Wadsworth-Emmons reaction. Synthetic derivatives were efficient in activating both hPPARα and hPPARγ as dual PPARα/γ agonists. These prenylated benzopyrans emerge as lead compounds that might be potentially useful for preventing cardiometabolic diseases.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

PPARs, Peroxisome proliferator-activated receptors; SAR, structure-activity relationships; DIBAL-H, Diisobutylaluminium hydride; TsN_3, Tosyl azide.

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(12)General procedure for the synthesis of alkylphosphonates: Synthesis of ethyl 2-(diethoxyphosphoryl)-2-ethoxyacetate (**8a**). Ethyl 2-diazo-2-(diethoxyphosphoryl) acetate (**7**, 379 mg, 1.503 mmol) in ethanol (900 µL) was stirred, and rhodium acetate (II) (6.66 mg, 0.015 mmol) in toluene (8 ml) was added to this solution. The mixture was stirred at 45ºC overnight. Usual work up followed by column chromatography purification (ethyl ether/ EtOAc 98:2) gave 162 mg of **8a** (40.2 %).

(13)Wadsworth Jr. W. S. Synthetic Applications of Phosphoryl-Stabilized Anions. Org. React. 1977, 25, 73−253.

(14)General procedure for Horner-Wadsworth-Emmons reaction: Synthesis of 6-benzyloxy-2-((8'-ethoxy-4'-methyl)ethylnona-3'*E*, 7'*Z*-dienoate)-2-methyl-dihydrobenzopyran (**15**): Ethyl 2-(diethoxyphosphoryl)-2-ethoxyacetate (**8a**) (0.156 mmol) in anhydrous THF (4 ml) at 0ºC under N_2 atmosphere was treated with 60% NaH (10.6 mg, 0.265 mmol) and the mixture was stirred for 1h at 0ºC. Then, a solution of aldehyde (59 mg, 0.156 mmol) in anhydrous THF (5 ml) was added and the mixture stirred overnight at room temperature. Usual work up followed by column chromatography purification (hexane/ EtOAc 80:20) yielded 64.5 mg of **15** (85 %) as a colorless oil: 1H NMR (300 MHz, $CDCl_3$): δ 7.36 (m, 5H, H-2" to 6"), 6.75 (dd, *J* = 7.1, 2.3 Hz, 1H, H-7), 6.72 (d, *J* = 7.1 Hz, 1H, H-8), 6.69 (d, *J* = 2.3 Hz, 1H, H-5), 6.23 (t, *J* = 7.4 Hz, 1H, H-7'), 5.16 (m, 1H, H-3'), 4.99 (s, 2H, OCH_2Ar), 4.21 (q, *J* = 7 Hz, 2H, $COOCH_2CH_3$), 3.85 (q, *J* = 7 Hz, 2H, OCH_2CH_3), 2.73 (t, *J* = 6.8 Hz, 2H, CH_2-4), 2.34 (q, *J* = 7.4 Hz, 2H, CH_2-6'), 2.09 (m, 4H, CH_2-2', CH_2-5'), 1.81 (m, 2H, CH_2-3), 1.62 (m, 2H, CH_2-1'), 1.60 (s, 3H, CH_3-10'), 1.30, 132 (2t, *J* = 7 Hz, 3H, OCH_2CH_3, $COOCH_2CH_3$), 1.27 (s, 3H, CH3-11'). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.6 (CO), 152.5 (C-6), 148.5 (C-8a), 145.4 (C-8'), 137.9 (C-1"), 134.5 (C-4'), 129.1 (CH-7'), 128.9, 128.2, 127.9 (CH-2" to CH-6"), 125.4 (CH-3'), 122.0 (C-4a), 116.4 (CH-7), 115.5 (CH-5), 114.7 (CH-8), 76.0 (C-2), 71.0 (OCH_2Ar), 68.4 (OCH_2CH_3), 61.1, ($COOCH_2CH_3$), 40.3 (CH_2-5'), 39.7 (CH2-1'), 31.1 (CH_2-3), 25.4. (CH_2-6'), 24.5 (CH_3-11'), 22.8 (CH_2-4), 22.6 (CH_2-2'), 16.2 (CH_3-10'), 15.8 ($COOCH_2CH_3$), 14.9 (OCH_2CH_3). HREIMS m/z 492.2869 [M]+ (492.2875 calcd. for $C_{31}H_{40}O_5$) (100%).

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