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Novel Human Metabolites of the Angiotensin-II Antagonist Tasosartan and Their Pharmacological Effects

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Abstract—Three novel metabolites of the angiotensin-II (A-II) receptor antagonist tasosartan have been identified in humans, and the syntheses and pharmacologic profiling of these metabolites are reported. Each metabolite bound the human A-II receptor with IC_{50}s between 20 and 45 nM. The in vivo effects of these compounds in attenuating the pressor response to angiotensin-II challenge in anesthetized rats were also investigated. An unsaturated diol metabolite exhibited in vivo efficacy at intravenous doses of 1 and 3 mg/kg, while the other metabolites, both carboxylic acids, had no significant effect at the same doses.

The development of drugs that act by modulating the renin–angiotensin system has produced a variety of agents over the past two decades.[1] The initial class of compounds inhibited the enzymatic production of angiotensin-II by acting on angiotensin converting enzyme (ACE inhibitors),[2] followed by compounds that blocked the angiotensin-II receptor.[3] Importantly, new data concerning the efficacy of these drugs continue to be developed. For example, the recently published HOPE trial indicated that ACE inhibition was unexpectedly associated with reduced myocardial infarction, and renoprotective effects of A-II antagonists independent of effects on blood pressure have been reported in diabetics.[4] These reports indicate that this class of compounds will continue to be a focus of both basic research and clinical studies.

We have previously reported the development of an angiotensin-II receptor antagonist, tasosartan, that was characterized preclinically using both in vitro and in vivo assays in rodent.[5] This agent progressed to clinical trials, where analysis of plasma from individuals treated with the compound revealed specific metabolites that were profiled pharmacologically in an earlier publication.[6] An example of one of these previously described metabolites is compound **2** (Fig. 1).

Recently, three additional novel human plasma metabolites were isolated. These human metabolites were oxidative products of tasosartan characterized by oxidation of one or both methyl groups with or without the dihydropyridone ring oxidation. Spectroscopic analysis of these metabolites supported the proposed structures. Two of the metabolites were assigned the mono-carboxylic acid structures (**3** and **4**) and the third was assigned the structure of the unsaturated diol (**5**) (Fig. 1). For structure confirmation, the purpose of this research report is to describe the syntheses of these metabolites, followed by their initial characterization in vitro and in vivo. In order to profile human metabolites, the in vitro analysis has specifically utilized a well-characterized human primary cell culture system.[7] In vivo profiling was performed using standard rat pressor assays.

Metabolite **3** was synthesized directly from tasosartan (**1**) in two steps (Scheme 1). Selenium dioxide oxidation in aqueous dioxane afforded the aldehyde (**6**). Further oxidation (benzeneseleninic acid, hydrogen peroxide) furnished the carboxylic acid (**3**). Contrary to that which was previously reported,[5] when the selenium dioxide oxidation reaction was carried out in anhydrous dioxane as a solvent, the main product isolated was the des-methyl pyridopyrimidine derivative (**7**) rather than the aldehyde (**6**). Compound **7** was erroneously reported to be the aldehyde **6**.[5]

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The 2-carboxylic acid metabolite (**4**) was prepared in three steps from 8-(4-bromobenzyl)-2-(hydroxymethyl)-4-methyl-5,8-dihydropyrido[2,3-*d*]pyrimidin-7(6*H*)-one (**8**)[6] (Scheme 2). Oxidation of **8** with MnO_2 afforded the carboxylic acid (**9**). Palladium-catalyzed coupling of the bromo derivative **9** with 2-[(2-*t*-butyl)-2*H*-tetrazol-5-yl]-phenylboronic acid furnished compound **10**. Deprotection of the tetrazole with hydrochloric acid and purification by HPLC[9] afforded metabolite **4**.

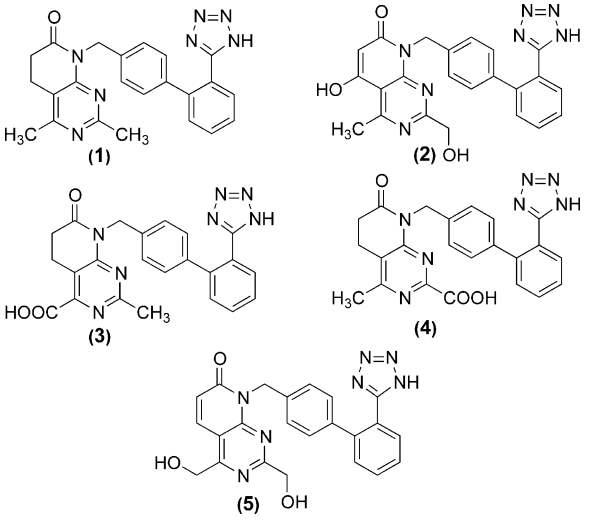
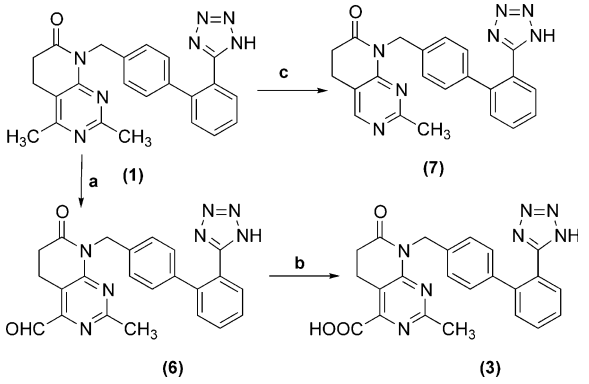
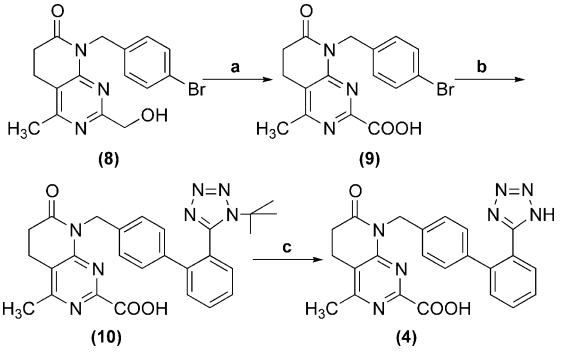


Figure 1. Structures of tasosartan™ and its human metabolites.



Scheme 1. Synthesis of metabolite **3**: (a) SeO_2, dioxane/water;[8] (b) $PhSeO_2H$, H_2O_2, THF; (c) SeO_2, dioxane.

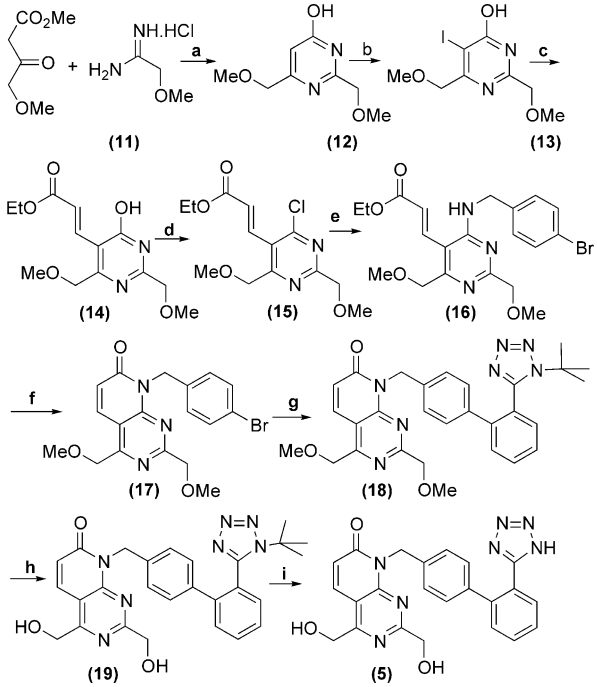


Scheme 2. Synthesis of metabolite **4**: (a) MnO_2, acetone; (b) $(Ph_3P)_4Pd$, Na_2CO_3, toluene, EtOH, H_2O, 2-[(2-*t*-butyl)-2*H*-tetrazol-5-yl]-phenylboronic acid; (c) HCl, heat.

The unsaturated bishydroxymethyl metabolite (**5**) was synthesized in eight steps (Scheme 3). Condensation of methoxymethyl acetamidine hydrochloride **11** with methyl methoxy acetoacetate yielded the pyrimidine derivative **12**. Iodination of **12** afforded the iodo pyrimidine **13** that was coupled with ethyl acrylate, under the Heck conditions to give the pyrimidinyl acrylate **14**. Conversion of the hydroxy group of **14** to the chloro group (**15**) with $POCl_3$ followed by reaction with 4-bromo-benzylamine afforded **16**. Cyclization of **16** at a temperature of 240 °C provided the pyridopyrimidinone **17**. Palladium-catalyzed coupling of **17** with 2-[(2-*t*-butyl)-2*H*-tetrazol-5-yl]-phenylboronic acid furnished compound **18**. Deprotection of the methoxy groups with BBr_3, followed by removal of the *t*-butyl protecting group of the tetrazole with hydrochloric acid, afforded metabolite **5**.

Tasosartan and its metabolites were tested for the inhibition of [^{125}I]A-II binding (AT_1 receptors). For the determination of angiotensin-II receptor binding by the compounds, studies were performed in cultured human preadipocytes. The test compounds were initially suspended in 100% DMSO at a concentration of 10 mM, then serially diluted with assay buffer to yield a final concentration from 10^{-7} to 10^{-9} M.[11] Results are shown in Table 1.

Rats weighing 343 ± 8 g were anesthetized with sodium pentobarbital (50 mg/kg, ip), placed in the supine position



Scheme 3. Synthesis of metabolite **5**:[10] (a) NaOEt, EtOH; (b) I_2, K_2CO_3, THF; (c) ethyl acrylate, $Pd(OAc)_2$, Et_3N, sealed tube, 130 °C; (d) $POCl_3$, benzene, heat; (e) 4-bromo-benzylamine, *n*-BuOH, Et_3N; (f) heat; (g) 2-[(2-*t*-butyl)-2*H*-tetrazol-5-yl]-phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, EtOH, H_2O, toluene, heat; (h) BBr_3, CH_2Cl_2; (i) HCl, heat.

Table 1. In vitro data for tasosartan and its metabolites

Compd	IC_{50}, nM[a]
1	38 ± 0.75
3	44 ± 0.30
4	23 ± 1.30
5	44 ± 0.54

[a]Determination of the IC_{50} for each compound was based upon linear regression curve fitting from data obtained from 2–3 separate experiments.

on a heating pad (K-model 100, Baxter Laboratories), and a longitudinal incision made on the neck to expose the right carotid artery and jugular vein. The jugular vein was cannulated with a saline filled polyethylene catheter (PE 10) and used for intravenous drug infusion. Arterial pressure and heart rate were monitored via a saline filled polyethylene catheter (PE 10) introduced into the carotid artery and connected to a P23 ID Statham/Gould pressure transducer. Subcutaneous needles were positioned in the limbs for ECG recordings. All data outputs were recorded on a Gould Model 6600 series recorder with a Po-ne-mah data acquisition system and displayed on a physiology platform CRS800W/CRS400W recorder.

The experimental protocol for assessing in vivo A-II antagonist activity involved compound dosing followed by measurement of the pressor response to A-II challenge. Each rat from an individual treatment group ($n = 3$–5) was initially dosed intravenously (iv) with either vehicle (2% ethanol in saline solution) or test compound (**1**, **3**, **4**, or **5**). The test compounds were administered initially at a dose of 0.3 mg/kg (iv) followed 2 min later by angiotensin-II challenge (3 μg/kg, iv), and the maximal increase in mean arterial pressure was recorded. Twenty minutes later, baseline pressure values were re-established and the next dose of test compound (1.0 mg/kg, iv) was administered followed by angiotensin-II challenge. This regimen was repeated for the 3.0 mg/kg dose for each test compound. All animals were euthanized at the conclusion of the protocol.

Each rat was randomly assigned to one of four drug treatment groups in an effort to evaluate the in vivo pressor response to an angiotensin-II challenge in the presence of one of the test compounds listed above. A dose–response protocol was established and the maximal change in arterial pressure following angiotensin-II was recorded for each dose of the compound. The results were represented as a percent change in maximal pressure recorded before and after the angiotensin-II challenge.

Tasosartan (1)

There was a 32 ± 0.2% increase in arterial pressure to an angiotensin-II challenge in the absence of any test compound in the group of rats assigned to this treatment group. Administration of tasosartan at doses of 1.0 and 3.0 mg/kg (iv) significantly ($p < 0.05$) attenuated the pressor response to angiotensin-II (Fig. 2, panel A).

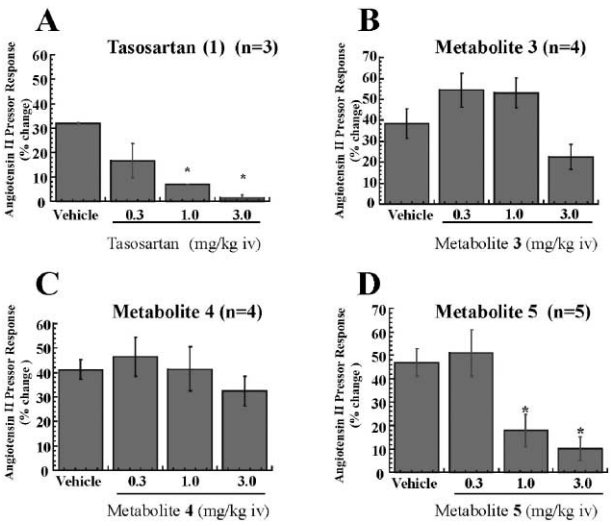


Figure 2. Pressor response to angiotensin-II administration in four groups of rats ($n = 3$–5 each). Each rat was subjected to four separate bolus injections of angiotensin-II following the introduction of either the vehicle or graded doses of tasosartan (**1**) (panel A), metabolite **3** (panel B), metabolite **4** (panel C) and metabolite **5** (panel D) (0.3, 1.0, or 3.0 mg/kg, iv). Data are presented as percent change (mean ± SEM) in the pressor response. *$p < 0.05$ versus the vehicle response in the respective treatment group.

Metabolite 3

There was a 38 ± 7% increase in arterial pressure to an angiotensin-II challenge in the absence of any test compound in the group of rats assigned to this treatment group. Administration of metabolite **3** at doses of 0.3, 1.0, or 3.0 mg/kg (iv) did not attenuate the pressor response to angiotensin-II (Fig. 2, panel B).

Metabolite 4

There was a 41 ± 4% increase in arterial pressure to an angiotensin-II challenge in the absence of any test compound in the group of rats assigned to this treatment group. Administration of metabolite **4** at doses of 0.3, 1.0, and 3.0 mg/kg (iv) failed to significantly attenuate the pressor response to angiotensin-II (Fig. 2, panel C).

Metabolite 5

There was a 47 ± 6% increase in arterial pressure to an angiotensin-II challenge in the absence of any test compound in the group of rats assigned to this treatment group. Administration of metabolite **5** at doses of 1.0 and 3.0 mg/kg (iv) significantly ($p < 0.05$) attenuated the pressor response to angiotensin-II. The 0.3 mg/kg dose did not alter the pressor response to the bolus injection of angiotensin-II (Fig. 2, panel D).

We describe the syntheses of three previously unidentified human metabolites of tasosartan (**1**) and their in vitro inhibition of A-II receptor binding in human cell cultures. While all three metabolites bound the A-II receptor, the 2-carboxylic acid metabolite **4** showed the greatest in vitro activity with an IC_{50} of 23 ± 1.3 nM.

The 4-carboxylic acid metabolite **3** and the bis-hydroxymethyl metabolite **5** were equipotent (IC_{50} = 44 ± 0.30 nM and IC_{50} = 44 ± 0.54 nM, respectively). However, in vivo testing of the three metabolites did not yield parallel results. At test doses of 1 and 3 mg/kg (iv), metabolite **5** produced a significant attenuation of the hypertensive response to A-II challenge similar to that observed for tasosartan. Metabolites **3** and **4** had no significant effect at the same doses. These data indicate that while all metabolites have similar in vitro inhibitory activity, only the unsaturated diol exhibits in vivo activity at the doses tested. Future studies examining the pharmacokinetics of each compound, including plasma stability and protein binding, need to be undertaken to better understand the diversity between in vitro and in vivo results.

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8. Synthesis of 2-methyl-7-oxo-8-{[2′-(2*H*-1,2,3,4-tetraazol-5-yl)[1,1′-biphenyl]-4-yl]methyl}-5,6,7,8-tetrahydropyrido[2,3-*d*]-pyrimidine-4-carboxaldehyde (**6**). 5,8-Dihydro-2,4-dimethyl-8-[(2′-(1*H*-tetrazol-5-yl)-[1,1′-biphenyl]-4-yl)methyl]pyrido[2,3-*d*]-pyrimidin-7(6*H*)-one (**1**) (1.2 g, 2.92 mmol) was added under nitrogen to selenium dioxide (0.33 g, 2.97 mmol) in 14 mL of dioxane and 0.25 mL of water and the mixture was heated at reflux for 4 h then cooled to room temperature. The mixture was filtered through Celite® and the filtrate was concentrated under reduced pressure to give 1.42 g of an orange glass. The material was purified by flash chromatography on a silica gel column using 18% THF in methylene chloride as the eluent. The fraction containing the desired material was concentrated under reduced pressure. The residue was stirred in ether and the solid was collected by filtration and dried to give the desired aldehyde as an off-white solid (0.75 g): mp 203–206 °C (decomposition); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.57 (s, 3H), 2.75 (t, J = 7.7 Hz, 2H), 3.31 (t, J = 7.7 Hz, 2H), 5.22 (s, 2H), 7.0 (d, J = 8.1 Hz, 2H), 7.22 (d, J = 8.1 Hz, 2H), 7.50–7.56 (m, 2H), 7.62–7.67 (m, 2H), 9.96 (s, 1H), 12.27 (br. s, 1H); IR (KBr), cm^{-1} 1700, 1715. HRMS [M + H]$^+$ Exact mass: 426.16730, Experimental: 426.16756. Anal. for $C_{23}H_{19}N_7O_2 \cdot 1/2H_2O$. Calcd: C, 63.59; H, 4.64; N, 22.57. Found: C, 63.83; H, 4.50; N, 22.45.
9. Compounds **3** and **4** were purified by reverse phase HPLC on a Primesphere C18 (5 × 25 cm) column with 0.1% TFA in acetonitrile as the mobile phase and detection at 254 nm.
10. Synthesis of 2,4-bis-hydroxymethyl-8-[2′(1H-tetrazol-5-yl)-biphenyl-4-ylmethyl]-8-[2′-(1*H*-tetrazol-5-yl)-bipheny-4-ylmethyl]-8*H*-pyrido[2,3-*d*]pyrimidin-7-one (**5**). **Step 1**: 2,6-Bis-(methoxymethyl)-4-pyrimidinol (**12**). To a solution of NaOEt/EtOH, made from EtOH (200 mL) and sodium (3.5 g), was added 1-methoxyacetamidine hydrochloride (13.7 g, 0.11 mol) and methyl methoxyethylacetoacetate (14.6 g, 0.1 mol). This mixture was heated to reflux for 4 h. The reaction mixture was cooled, and acidified with hydrochloric acid to a pH of ~6. The solvent was evaporated under vacuum. The residue was extracted with hot ethanol (3×300 mL). The ethanolic extracts were combined and evaporated to dryness. The product was purified by flash chromatography (silica gel; MeOH/$CHCl_3$, 3:97) to afford 15.8 g (85%) of a white solid. Mp 99–100 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.32 (s, 3H), 3.36 (s, 3H), 4.22 (s, 2H), 4.24 (s, 2H), 6.15 (s, 1H), 12.35 (br. s, 1H); IR (KBr), cm^{-1} 1680; MS (+ESI, [M + H]$^+$) m/z 185. Anal. for $C_8H_{12}N_2O_3$. Calcd: C, 52.17; H, 6.57; N, 15.21. Found: C, 52.17; H, 6.32; N, 15.14. **Step 2**: 5-Iodo-2,6-bis(methoxy-methyl)-4-pyrimidinol (**13**). To a well-stirred solution of **12** (8.28 g, 0.045 mol) in THF (300 mL) were added iodine (22.86 g, 0.09 mol) and potassium carbonate (13.0 g, 0.095 mol). The mixture was heated at reflux for 6 h then cooled to ambient temperature. The mixture was neutralized with hydrochloric acid and solvent evaporated. The product was purified by flash chromatography (silica gel; MeOH/$CHCl_3$, 3:97) to afford 3.3 g of a white solid. Mp 112–114 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.33 (s, 3H), 4.27 (s, 2H), 4.39 (s, 2H), 12.80 (br s, 1H); IR (KBr), cm^{-1} 1650; MS (+ESI, [M + H]$^+$) m/z 311. Anal. for $C_8H_{11}IN_2O_3$. Calcd: C, 30.99; H, 3.58; N, 9.03. Found: C, 30.83; H, 3.38; N, 8.86. **Step 3**: Ethyl (*E*)-3-[4-hydroxy-2,6-bis(methoxy-methyl)-5-pyrimidinyl]-2-propenoate (**14**). A mixture of the iodo-pyrimidine (**13**) (3.1 g, 0.01 mol), ethyl acrylate (2.2 mL, 0.02 mol), palladium acetate (0.1 g) and triethyl amine (10 mL) was heated under nitrogen in a sealed tube at 130 °C for 45 min. The mixture was cooled to ambient temperature, diluted with chloroform (300 mL), and filtered through Celite®. The filtrate was evaporated to dryness and the product was purified by flash chromatography (silica gel; 20–40% ethyl acetate in hexane) to afford 1.8 g (64%) of an off-white solid. Mp 162–163 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25 (t, J = 7.2 Hz, 3H), 3.31 (s, 3H), 3.34 (s, 3H), 4.17 (q, J = 7.2 Hz, 2H), 4.31 (s, 2H), 4.46 (s, 2H), 7.26 (d, J = 15.6 Hz, 1H), 7.69 (d, J = 15.6 Hz, 1H), 12.95 (br. s, 1H); IR (KBr), cm^{-1} 1700, 1670, 1625; MS (+ESI, [M + H]$^+$) m/z 283. Anal. for $C_{13}H_{18}N_2O_5$. Calcd: C, 55.31; H, 6.43; N, 9.92. Found: C, 55.24; H, 6.15; N, 9.73. **Step 4**: Ethyl (*E*)-3-[4-chloro-2,6-bis-(methoxy-methyl)-5-pyrimidinyl]-2-propenoate (**15**). A mixture of the hydroxy-pyrimidine (**14**) (1.2 g, 0.004 mol), phosphorus oxychloride (1.0 mL), and benzene (20 mL) was heated at reflux for 2 h. The mixture was cooled to ambient temperature, poured over ice, neutralized with sodium bicarbonate, and

extracted with ethyl acetate. The organic phase was washed with water, dried and evaporated. Purification by flash chromatography (silica gel; 20% ethyl acetate in hexane) afforded 0.8 g (67%) of an oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.26 (t, J = 7 Hz, 3H), 3.31 (s, 3H), 3.38 (s, 3H), 4.22 (q, J = 7 Hz, 2H), 4.54 (s, 2H), 4.58 (s, 2H), 6.51 (d, J = 16.24 Hz, 1H), 7.65 (d, J = 16.24 Hz, 1H); IR (KBr), cm^{-1} 1720; MS (+ESI, $[M+H]^+$) m/z 301. Anal. for $C_{13}H_{17}ClN_2O_4 \cdot 1/4H_2O$. Calcd: C, 51.15; H, 5.78; N, 9.18. Found: C, 51.32; H, 5.53; N, 9.25. **Step 5**: Ethyl *(E)*-3[4-(4-bromo-benzylamino)-2,6-bis(methoxymethyl)-5-pyrimidinyl]-2-propenoate (**16**). A mixture of the chloropyrimidine (**15**) (0.6 g, 0.002 mol), 4-bromo-benzylamine (0.4 g, 0.004 mol), triethylamine (2 mL), and butanol (25 mL) was heated at reflux for 2 h. The solvent was evaporated under vacuum. The residue was dissolved in ethyl acetate (100 mL) and water (50 mL). The organic phase was washed with water, dried and evaporated to dryness. The product was purified by flash chromatography (silica gel; 40–50% ethyl acetate in hexane) to afford 0.85 g (94%) of an oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.27 (t, J = 7.14 Hz, 3H), 3.27 (s, 3H), 3.31 (s, 3H), 4.20 (q, J = 7.14 Hz, 2H), 4.28 (s, 2H), 4.29 (s, 2H), 4.57 (d, J = 5.92 Hz, 2H), 6.38 (d, J = 16.04 Hz, 1H), 7.29 (d, J = 8.56 Hz, 1H), 7.47 (d, J = 8.56 Hz, 2H), 7.63 (d, J = 16.04 Hz, 1H), 7.88 (t, J = 5.92 Hz, 1H); IR (KBr), cm^{-1} 3350, 1710; MS (+ESI, $[M+H]^+$) m/z 450/452. Anal. for $C_{20}H_{24}BrN_3O_4$. Calcd: C, 53.34; H, 5.37; N, 9.33. Found: C, 52.95; H, 5.41; N, 9.16. **Step 6**: 8-(4-Bromo-benzyl)-2,4-bis(methoxymethyl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (**17**). The pyrimidinyl-propenoate (**16**) was heated under vacuum at 240 °C for 4 h. The mixture was cooled to ambient temperature and the product was purified by flash chromatography (silica gel; 60% ethyl acetate in hexane) to afford 0.4 g (60%) of an off-white solid. Mp 113–115 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.36 (s, 3H), 3.37 (s, 3H), 4.62 (s, 2H), 4.79 (s, 2H), 5.51 (s, 2H), 6.81 (d, J = 9.64 Hz, 1H), 7.32 (d, J = 8.34 Hz, 2H), 7.48 (d, J = 8.34 Hz, 2H), 8.26 (d, J = 9.64 Hz, 1H); IR (KBr), cm^{-1} 1675, 1570; MS (+ESI, $[M+H]^+$) m/z 404/406. Anal. for $C_{18}H_{18}BrN_3O_3$. Calcd: C, 53.48; H, 4.49; N, 10.39. Found: C, 53.35; H, 4.38; N, 10.10. **Step 7**: 8-({2′-[2-(*tert*-Butyl)-2H-1,2,3,4-tetrazol-5-yl] [1,1′-biphenyl]-4-yl}methyl)-2,4-bis-(methoxy methyl) pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (**18**). A mixture of **17** (0.81 g, 0.0020 mol), 2-[(2-*tert*-butyl)-*2H*-tetrazol-5-yl]-phenylboronic acid (0.59 g, 0.0024 mol), tetrakis-triphenylphosphine palladium (150 mg), sodium carbonate (0.8 g), ethanol (3 mL), toluene (20 mL) and water (2 mL) was heated at reflux for 18 h. The solvent was evaporated. The residue was treated with ethyl acetate (200 mL) and filtered. The filtrate was evaporated to dryness and the residue was purified by flash chromatography (silica gel; 60% ethyl acetate in hexane) to afford 0.92 g (88%) of an oil. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.32 (s, 9H), 3.36 (s, 3H), 3.37 (s, 3H), 4.61 (s, 2H), 4.79 (s, 2H), 5.54 (s, 2H), 6.80 (d, J = 9.84 Hz, 1H), 6.98 (d, J = 8.24 Hz, 2H), 7.31 (d, J = 8.24 Hz, 2H), 7.43 (d, J = 7.68 Hz, 1H), 7.51 (t, 5.92 Hz, 1H), 7.58 (t, J = 5.92 Hz, 1H), 7.77 (d, J = 7.60 Hz, 1H), 8.23 (d, J = 10.0 Hz, 1H); IR (KBr), cm^{-1} 1670, 1570; MS (+APCI, $[M+H]^+$) m/z 526. Anal. for $C_{29}H_{31}N_7O_3 \cdot H_2O$. Calcd: C, 64.07; H, 6.12; N, 18.04. Found: C, 63.67; H, 5.73; N, 18.12. **Step 8**: 8-({2′-[2-(*tert*-Butyl)-2H-1,2,3,4-tetrazol-5-yl][1,1′-biphenyl]-4-yl}methyl)-2,4-bis(hydroxymethyl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (**19**). Boron tribromide (6 mL of 1 M solution in methylene chloride, 0.0060 mol) was added slowly to the solution of **18** (0.8 g, 0.0015 mol) in methylene chloride (50 mL) at ambient temperature. The mixture was stirred for 3 h. Methanol (5 mL) was added dropwise. The mixture was stirred for 30 min and water (2 mL) was added. The solvent was evaporated and the product was purified by flash chromatography (silica gel; 1–3% MeOH in $CHCl_3$) to afford 0.6 g (80%) of a white solid. Mp 107–109 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.34 (s, 9H), 4.64 (d, J = 6.12 Hz, 2H), 4.84 (d, J = 5.72 Hz, 2H), 5.36 (t, 6.26 Hz, 1H), 5.58 (s, 2H), 5.68 (t, J = 5.92 Hz, 1H), 6.75 (d, J = 9.88 Hz, 1H), 6.97 (d, J = 8.32 Hz, 2H), 7.34 (d, J = 8.36 Hz, 2H), 7.43 (d, 7.44 Hz, 1H), 7.51 (t, J = 7.48 Hz, 1H), 7.58 (t, J = 7.48 Hz, 1H), 7.77 (d, J = 7.68 Hz, 1H), 8.31 (d, J = 9.68 Hz, 1H); IR (KBr), cm^{-1} 1670, 1565; MS (+APCI, $[M+H]^+$) m/z 498. Anal. for $C_{27}H_{27}N_7O_3$. Calcd: C, 65.18; H, 5.47; N, 19.71. Found: C, 64.91; H, 5.45; N, 19.36. **Step 9**: 2,4-Bis(hydroxymethyl)-8-{[2′-(1H-1,2,3,4-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]-methyl}pyrido[2,3-*d*]pyrimidin-7(8H)-one hydrochloride (**5**). A mixture of **19** (0.2 g, 0.0004 mol) and hydrochloric acid (10 mL) was heated at reflux for 2 h. The mixture was cooled to ambient temperature, diluted with water (30 mL), and filtered. Evaporation of the solvent under vacuum and drying of the residue under high vacuum afforded the product as a solid (0.162 g). Mp 195–197 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.64 (s, 2H), 4.84 (d, 2H), 5.58 (s, 2H), 6.75 (d, J = 9.64, 1H), 6.99 (d, J = 8.32 Hz, 2H), 7.29 (d, J = 8.32 Hz, 2H), 7.44–7.58 (m, 2H), 7.60–7.66 (m, 2H), 8.32 (d, J = 9.8, 8 Hz, 1H); IR (KBr), cm^{-1} 16650, 1570; MS (+ESI, $[M+H]^+$) m/z 442. Anal. for $C_{23}H_{19}N_7O_3 \cdot HCl$. Calcd: C, 57.80; H, 4.22; N, 20.52. Found: C, 57.47; H, 4.16; N, 20.13.

11. Between passages 2 and 5, preadipocytes from the subcutaneous depot (Zen-Bio, Research Triangle Park, NC, USA) were plated in 24-well plates at a density of 25,000 cells/well. Cells were maintained in Medium 199 supplemented with 10% heat-inactivated fetal bovine serum and 1% antibiotic-antimycotic (Life Technologies, Grand Island, NY, USA). The experiment was initiated by aspirating the medium and rinsing the cells twice with phenol red-free Medium 199. The binding assay was then begun by adding 160 μL of binding buffer (50 mM Tris, 5 mM $MgCl_2$, 0.25% bovine serum albumin, pH 7.4; Sigma, St. Louis, MO), 20 μL of 10 μM unlabelled A-II (nonspecific binding), 20 μL of buffer (total binding), or 20 μL of test compound. The cells were incubated at room temperature for 10 min, followed by addition of 20 μL of ^{125}I-[Sar^1,-Ile^8]A-II (Amersham, Arlington Heights, IL, USA). The culture plate was shaken gently for 1 h at room temperature, followed by aspiration of the medium. Each well was rinsed twice with 300 μL of the assay buffer, followed by addition of 100 μL of 0.5 M NaOH/0.5% Triton to solubilize the cells. The radioactivity from each well was then determined in an appropriate detector.