Supporting Information

Title:

Design, synthesis and in vitro testing of α-methylacyl-CoA racemase inhibitors

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Contents:

Methyl 2-trifluoromethyltetradec-2-enoate (9) (*E*)-isomer: δ ^{1}H (400MHz, $CDCl_3$) 0.88 (3H, t, J = 7.0 Hz, H-14), 1.22-1.38 (16H, m, H-6 to H-13), 1.41-1.52 (2H, m, H-5), 2.56-2.62 (2H, m, H-4), 3.82 (3H, s, CO_2Me), 6.84 (1H, tq, $J_{3,4}$ = 7.4 Hz, $J_{H\text{-}F}$ = 1.5 Hz, H-3); δ ^{13}C (100MHz, $CDCl_3$) 14.5 (C-14), 23.0-32.3 (C-4- to C-13), 52.3 ($CO_2\underline{Me}$), 128.9 (C-2), 151.6 (C-3), 169.1 (C-1); (*Z*)-isomer: δ ^{1}H (400MHz, $CDCl_3$) 0.88 (3H, t, J = 7.0 Hz, H-14), 1.22-1.38 (16H, m, H-6 to H-13), 1.41-1.52 (2H, m, H-5), 2.42-2.48 (2H, m, H-4), 3.82 (3H, s, CO_2Me), 7.21 (1H, td, $J_{3,4}$ = 7.6 Hz, $J_{H\text{-}F}$ = 0.9 Hz, H-3); δ ^{13}C (100MHz, $CDCl_3$) 14.5 (C-14), 23.0-32.3 (C-4 to C-13), 52.3 ($CO_2\underline{Me}$), 128.9 (C-2), 154.5 (C-3), 169.1 (C-1); ν_{max}/cm^{-1} 1717 (C=O), 1651 (C=C); HRMS calcd for $C_{16}H_{35}F_3O_2N$ ($M+NH_4^+$) 236.2307; found 326.2301

methyl 2-trifluoromethyltetradecanoate Rf = 0.69 Et_2O:hexane (1:4); δ ^{1}H (400MHz, $CDCl_3$) 0.88 (3H, t, J = 7.0 Hz, H-14), 1.22-1.38 (20H, m, H-4 to H-13), 1.72-1.79 (1H, m, H-3), 1.83-1.91 (1H, m, H-3), 3.05-3.14 (1H, m, H-2), 3.77 (3H, s, CO_2Me); δ ^{13}C (100MHz, $CDCl_3$) 14.4 (C-14), 23.0 (C-3), 26.4-32.3 (C-4 to C-13), 50.8 (C-2), 52.3 ($CO_2\underline{Me}$), 128.1 (CF_3), 168.5 (C-1) ; ν_{max}/cm^{-1} 1756 (C=O); HRMS calcd for $C_{16}H_{33}F_3O_2N$ ($M+NH_4^+$) 328.2463; found 328.2460.

2-Difluoromethylenepentadecanoic acid (10)[15] δ ^{1}H (400MHz, $CDCl_3$) 0.88 (3H, t, J = 7.0 Hz, H-15), 1.22-1.36 (20H, m, H-5 to H-14), 1.41-1.52 (2H, m, H-4), 2.21-2.29 (2H, m, H-3); δ ^{13}C (100MHz, $CDCl_3$) 14.4 (C-15), 23.0-29.6 (C-4 to C-14), 32.2 (C-3), 168.3 (C-1); HRMS calcd for $C_{16}H_{28}F_2O_2$ ($M+H^+$) 290.1861; found 290.1856.

***N*-(2*S*,3*R*,4'*S*)-(3-hydroxy-2-methylhexadecanoyl)-4'-isopropyloxazolidin-2'-one (12)** δ ^{1}H (400MHz, $CDCl_3$) 0.88 (3H, t, J = 6.9 Hz, H-16), 0.89 (3H, d, J = 6.8 Hz, H-7′), 0.92 (3H, d, J = 7.0 Hz, H-8′), 1.24 (3H, d, J = 6.9 Hz, 2-Me), 1.22-1.36 (22H, m, H-5 to H-15), 1.58-1.64 (2H, m, H-4),

2.30-2.38 (1H, m, H-6′), 3.76 (1H, dq, $J_{H\text{-}Me}$ = 7.16 Hz, $J_{2,3}$ = 2.52 Hz (*syn*), H-2), 3.89-3.94 (1H, m, H-3), 4.22 (2H, dd, $J_{5',4'}$= 9.2 Hz, $J_{5',5'}$ = 3.1 Hz, H-5′), 4.44-4.49 (1H, m, H-4′); ^{13}C (100MHz, $CDCl_3$) 11.1 (C-2Me), 14.5 (C-16), 15.1 (C-8′), 18.3 (C-7′), 23.1-32.3 (C-6′, C-4 to C-15), 42.4 (C-2), 71.6 (C-3), 58.6 (C-4′), 63.8 (C-5′), 154.1 (C-2′), 173.1 (C-1); HRMS calcd for $C_{23}H_{43}NO_4Na$ ($M+Na^+$) 420.3080; found 420.3090

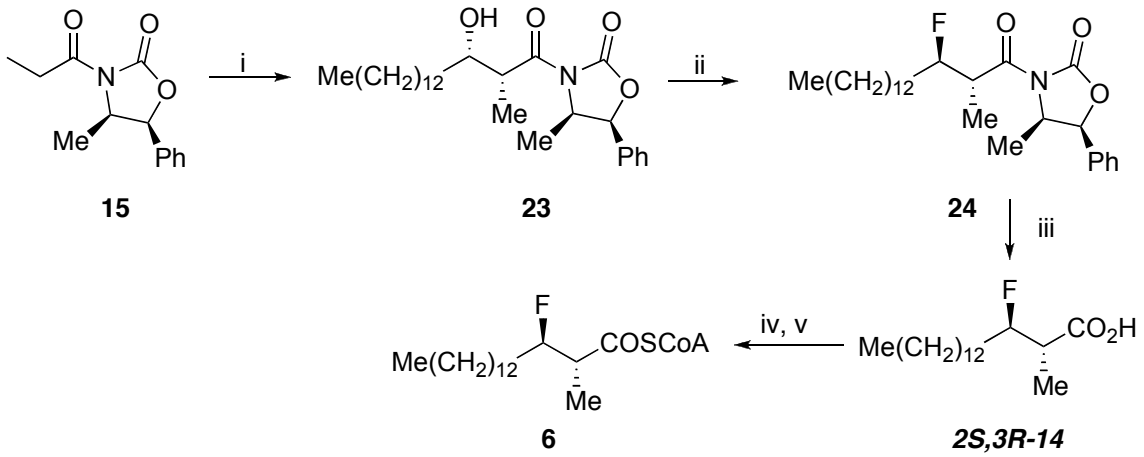
***N*-(2*R*,3*S*,4′*S*)-(3-fluoro-2-methylhexadecanoyl)-4′-isopropyl-oxazolidin-2′-one (13)**

δ ^{1}H (400MHz, $CDCl_3$) 0.88 (3H, t, *J* = 6.9 Hz, H-16), 0.89 (3H, d, *J* = 6.8 Hz, H-7′), 0.92 (3H, d, *J* = 7.0 Hz, H8′), 1.16 (3H, d, *J* = 6.9 Hz, 2-Me), 1.22-1.38 (22H, m, H-5 to H-15), 1.56-1.62 (2H, m, H-4), 2.30-2.38 (1H, m, H-6′), 4.12-4.18 (1H, m, H-2), 4.22-4.29 (1H, m, H-5′), 4.44-4.49 (1H, m, H-4′), 4.73 (1H, m, H-3); δ ^{13}C (100MHz, CDCl3) 14.2 (2-Me), 14.5 (C-16), 15.2 (C-8′), 18.3 (C-7′), 23.0-32.3 (C-6′, C-4 to C-15), 42.3 (C-2, d, *J* = 21 Hz), 59.0 (C-4′), 63.9 (C-5′), 94.3 (C-3, d, *J* = 168 Hz), 154.1 (C-2′), 173.1 (C-1); HRMS calcd for $C_{23}H_{42}FNO_3Na$ ($M+Na^+$) 422.3048; found 422.3046

(2*R*,3*S*)-3-Fluoro-2-methylhexadecanoic acid (14)

δ ^{1}H (400MHz, $CDCl_3$) 0.88 (3H, t, *J* = 7.0 Hz, H-16), 1.21 (3H, d, *J* = 7.1 Hz, 2-Me), 1.23-1.34 (22H, m, H-5 to H-15), 1.56-1.64 (2H, m, H-4), 2.78 (1H, ddq, $J_{H\text{-}F}$ = 12 Hz, $J_{H\text{-}Me}$ = 7.2 Hz, $J_{2,3}$ = 7.2 Hz (*anti*), H-2), 4.60-4.74 (1H, dm, $J_{H3\text{-}F}$ = 47.3 Hz, H-3); δ ^{13}C (100MHz, $CDCl_3$) 14.1 (2-Me), 14.5 (C-16), 21.0-31.9 (C-4 to C-15), 44.1 (C-2, d, *J* = 21 Hz), 94.4 (C-3, d, *J* = 171 Hz), 171.1 (C-1); HRMS calcd for $C_{17}H_{37}FO_2N$ ($M+NH_4^+$) 306.2808; found 306.2815.

Synthetic route to inhibitor 6 starting with auxiliary 15 (compounds 23 - 2*S*,3*R*-14 not shown in paper).



***N*-(*2R*,3*S*,4′*R*,5′*S*)-3-hydroxy-2-methylhexadecanoyl)-4′-methyl-5′-phenyloxazolidin-2′-one (23)**

(4*R*,5*S*)-4-Methyl-5-phenyl-3-propionyloxazolidin-2-one **(15)** (0.65ml, 3.27mmol, 1.4eq) was reacted according to the procedure described to make compound **12** to yield the title compound **(23)** as a clear oil (690mg, 66%), Rf = 0.19 Et_2O:hexane (1:1); δ ^{1}H (400MHz, $CDCl_3$) 0.88 (3H, t, *J* = 6.9 Hz, H-16), 0.90 (3H, d, *J* = 7.1 Hz, H-6′), 1.24 (3H, d, *J* = 6.9 Hz, 2-Me), 1.22-1.38 (22H, m, H-5 to H-15), 1.58-1.64 (2H, m, H-4), 3.77 (1H, dq, $J_{H\text{-}Me}$ 8.7 Hz, $J_{2,3}$ = 2.3 Hz (*syn*), H-2), 3.93-3.99 (1H, m, H-3), 4.72-4.78 (1H, m, H-4′), 5.68 (1H, d, *J* = 7.1 Hz, H-5′), 7.30-7.45 (5H, m, aromatics); ^{13}C (100MHz, $CDCl_3$) 10.5 (C-2Me), 14.5 (C-6′), 14.6 (C-16), 23.1-32.3 (C-4 to C-15), 42.5 (C-2), 55.1 (C-4′), 71.9 (C-3), 79.3 (C-5′), 129.1 (aromatics), 153.1 (C-2′), 177.9 (C-1); HRMS calcd for $C_{27}H_{43}NO_4Na$ ($M+Na^+$) 468.3083; found 468.3090.

***N*-(*2S*,3*R*,4′*R*,5′*S*)-(3-fluoro-2-methylhexadecanoyl)-4′-methyl-5′-phenyloxazolidin-2′-one (24).**

N-(*2R*,3*S*,4′*R*,5′*S*)-3-Hydroxy-2-methylhexadecanoyl)-4′-methyl-5′-phenyloxazolidin-2′-one(**23**) (180mg, 0.41mmol) was reacted according to the procedure described to make compound 13 to yield

the title compound (24) as a clear oil (34.3mg, 20%, Rf = 0.72 Et_2O:hexane (1:1); d ^1H (400MHz, $CDCl_3$) 0.88 (3H, t, J = 6.9 Hz, H-16), 0.90 (3H, d, J = 7.1 Hz, H-6′), 1.16 (3H, d, J = 6.9 Hz, 2-Me), 1.22-1.38 (22H, m, H-5 to H-15), 1.56-1.62 (2H, m, H-4), 4.10 (1H, ddq, $J_{H\text{-}F}$ = 5.23 Hz, $J_{H\text{-}Me}$ = 6.96 Hz, $J_{2,3}$ = 8.6 Hz (*anti*), H-2), 4.68-4.84 (1H, dm, $J_{H\text{-}F}$ = 48.1Hz, H-3), 4.78-4.82 (1H, m , H4′), 5.68 (1H, d, J = 7.1 Hz, H-5′), 7.39 (5H, m, aromatics); d ^{13}C (100MHz, $CDCl_3$) 13.9 (2-Me), 14.1 (C-6′), 14.4 (C-16), 22.7-31.9 (C-4 to C-15), 42.1 (C-2, d, J = 21 Hz), 55.1 (C-4′), 79.0 (C-5′), 94.8 (C-3, d, J = 169 Hz), 125.8 (aromatics), 152.7 (C-2′), 174.5 (C-1); HRMS calcd for $C_{27}H_{43}FNO_3$ ($M+H^+$) 448.1184; found 448.1180

(2*S*,3*R*)-3-fluoro-2-methylhexadecanoic acid (14).

N-(*2S*,3*R*,4′*R*,5′*S*)-3-Fluoro-2-methylhexadecanoyl)-4′-methyl-5′-phenyloxazolidin-2′-one**(24)** (34.3mg, 76.7μmol) was reacted according to the procedure described to make compound **14** to yield the title compound **(2*S*,3*R*)-(14)** as a clear oil (21mg, 95%), Rf = 0.33 Et_2O:hexane (1:1); δ ^1H (400MHz, $CDCl_3$) 0.88 (3H, t, J = 7.0 Hz, H-16), 1.21 (3H, d, J = 7.1 Hz, 2-Me), 1.23-1.36 (22H, m, H-5 to H-15), 1.56-1.64 (2H, m, H-4), 2.72-2.82 (1H, m, H-2), 4.62-4.73 (1H, dm, $J_{H\text{-}F}$ = 47.2 Hz, H-3); δ ^{13}C (100MHz, $CDCl_3$) 13.9 (2-Me), 14.1 (C-16) 22.7-31.9 (C-4 to C-15), 44.4 (C-2, d, J = 22 Hz), 95.3 (C-3, d, J = 171 Hz), 179.6 (C-1); HRMS calcd for $C_{17}H_{32}O_2F$ ($M+H^-$) 287.2393; found 287.2386

(2*S*,3*R*)-3-fluoro-2-methylhexadecanoyl-CoA (6).

(2*S*,3*R*)-3-Fluoro-2-methylhexadecanoic acid (3.4mg, 11.81μmol) (**14**) was reacted according to the procedure described to make compound **(5)** to yield the title compound **(6)** (1.89μmol, 27% yield-calculated from UV absorbtion of a specific concentration) UV / vis- absorbtion maxima at 260nm; HRMS calcd for $C_{38}H_{63}FN_7O_{17}P_3S$ ($M+2H^{2-}$) 517.6667; found 517.6689

All intermediates for the synthesis of compound (7)

methyl cholate δ ^{1}H (400MHz, $CDCl_3$) 0.74 (3H, s, 18-Me), 0.94 (3H, s, 19-Me), 0.99-1.02 (1H, m, H-1), 1.04 (3H, d, *J* = 6.5 Hz, 21-Me), 1.10-1.14 (1H, m, H-15), 1.32-1.50 (7H, m, H-2, H-4, H-5, H-15, H-16, H-20, H-22), 1.52-1.70 (5H, m, H-2, H-6, H-8, H-11, H-17), 1.74-2.04 (7H, m, H-1, H-6, H-9, H-11, H-14, H-16, H-22), 2.18-2.40 (4H, m, H-4, H-23), 3.35-3.42 (1H, m, H-3), 3.67 (3H, s, CO_2Me), 3.80-3.83 (1H, m, H-7), 3.97 (1H, t, *J* = 2.5 Hz, H-12); δ ^{13}C (100MHz, CDCl3) 13.3 (C-18), 18.1 (C-19), 18.2 (C-21), 23.1-32.7 (C-1, C-2, C-11, C-15, C-16, C-20, C-22, C-23), 36.2-48.5 (C-4, C-5, C-6, C-8, C-9, C-10, C-13, C-14, -C17), 52.3 (CO_2Me), 69.4 (C-7), 73.3 (C-3), 74.4 (C-12), 178.7 (C-24); HRMS calcd $C_{25}H_{42}O_5$ ($M+H^+$) 422.3448; found 422.3441

methyl (3α,7α,12α)-*tris*(methoxyethoxymethyl) cholate δ ^{1}H (400MHz, $CDCl_3$) 0.68 (3H, s, 18-Me), 0.89 (3H, s, 19-Me), 0.99-1.02 (1H, m, H-1), 1.04 (3H, d, *J* = 6.5 Hz, 21-Me), 1.10-1.14 (1H, m, H-15), 1.32-1.50 (7H, m, H-2, H-4, H-5, H-15, H-16, H-20, H-22), 1.52-1.70 (5H, m, H-2, H-6, H-8, H-11, H-17), 1.74-2.04 (7H, m, H-1, H-6, H-9, H-11, H-14, H-16, H-22), 2.16-2.20 (2H, m, H-4), 2.32-2.40 (2H, m, H-23), 3.40 (9H, s, OMe), 3.53-3.55 (1H, m, H-3), 3.55-3.58 (6H, m, H-3′), 3.63-3.65 (1H, m, H-7), 3.66 (3H, s, CO_2Me), 3.68-3.72 (6H, m, H-2′), 3.80-3.84 (1H, m, H-12), 4.76-4.84 (6H, m, H-1′); δ ^{13}C (100MHz, $CDCl_3$) 12.9 (C-18), 18.1 (C-19), 23.8 (C-21), 23.4-32.7 (C-1, C-2, C-11, C-15, C-16, C-20, C-22, C-23), 36.2-48.5 (C-4, C-5, C-6, C-8, C-9, C-10, C-13, C-14, C-17), 51.9 (CO_2Me), 59.4 (OMe), 67.0 (C-2′), 72.2 (C-3′), 74.9 (C-3), 77.2 (C-7), 79.9 (C-12), 94.5 (C-1′), 175.1 (C-24); HRMS calcd. For $C_{37}H_{66}O_{11}Na$ ($M+Na^+$) 709.4478 found; 709.4503

(3α,7α,12α)-*tris*(methoxyethoxymethyl)-5β-cholestan-24-ol δ ^{1}H (400MHz, $CDCl_3$) 0.68 (3H, s, 18-Me), 0.89 (3H, s, 19-Me), 0.99-1.02 (1H, m, H-1), 1.04 (3H, d, *J* = 6.5 Hz , 21-Me), 1.10-1.14 (1H, m, H-15), 1.32-1.50 (9H, m, H-2, H-4, H-5, H-15, H-16, H-20, H-22, H-23), 1.52-1.70 (5H, m,

H-2, H-6, H-8, H-11, H-17), 1.74-2.04 (7H, m, H-1, H-6, H-9, H-11, H-14, H-16, H-22), 2.18-2.26 (2H, m, H-4), 3.40 (9H, s, OMe), 3.53-3.55 (1H, m, H-3), 3.55-3.58 (6H, m, H-3′), 3.58-3.62 (2H, m, H-24), 3.63-3.65 (1H, m, H-7), 3.68-3.72 (6H, m, H-2′), 3.80-3.84 (1H, m, H-12), 4.76-4.84 (6H, m, H-1′); δ ^{13}C (100MHz, $CDCl_3$) 12.9 (C-18), 18.1 (C-19), 23.8 (C-21), 23.4-32.7 (C-1, C-2, C-11, C-15, C-16, C-20, C-22, C-23), 36.2-48.5 (C-4, C-5, C-6, C-8, C-9, C-10, C-13, C-14, C-17), 59.1 (OMe), 63.5 (C-24), 66.7 (C-2′), 71.8 (C-3′), 74.9 (C-3), 77.2 (C-7), 79.9 (C-12), 94.5 (C-1′); HRMS calcd for $C_{36}H_{66}O_{10}Na$ ($M+Na^+$) 681.4542; found 681.4554.

(3α,7α,12α)-*Tris*(methoxyethoxymethyl)-5β-cholestan-24-al (17) δ ^{1}H (400MHz, $CDCl_3$) 0.68 (3H, s, 18-Me), 0.89 (3H, s, 19-Me), 0.99-1.01 (1H, m, H-1), 1.04 (3H, d, *J* = 6.5 Hz, 21-Me), 1.10-1.14 (1H, m, H-15), 1.32-1.50 (7H, m, H-2, H-4, H-5, H-15, H-16, H-20, H-22), 1.52-1.70 (5H, m, H-2, H-6, H-8, H-11, H-17), 1.74-2.04 (7H, m, H-1, H-6, H-9, H-11, H-14, H-16, H-22), 2.18-2.28 (2H, m, H-4), 2.37-2.44 (2H, m, H-23), 3.40 (9H, s, OMe), 3.53-3.55 (1H, m, H-3), 3.56-3.59 (6H, m, H-3′), 3.63-3.65 (1H, m, H-7), 3.68-3.72 (6H, m, H-2′), 3.80-3.84 (1H, m, H-12), 4.76-4.84 (6H, m, H-1′), 9.77 (1H, t, *J* = 1.7 Hz, H-24); δ ^{13}C (100MHz, $CDCl_3$) 12.9 (C-18), 18.1 (C-19), 23.8 (C-21), 23.4-32.7 (C-1, C-2, C-11, C-15, C-16, C-20, C-22), 36.2-48.5 (C-4, C-5, C-6, C-8, C-9, C-10, C-13, C-14, C-17, C-23), 59.4 (OMe), 67.0 (C-2′), 72.2 (C-3′), 74.9 (C-3), 77.2 (C-7), 79.9 (C-12), 94.5 (C-1′), 202.6 (C-24); HRMS calcd for $C_{36}H_{64}O_{10}Na$ ($M+Na^+$) 679.4391; found 679.4397.

***N*-(24*R*,25*S*,4′*S*)-(3α,7α,12α)-*tris*(methoxyethoxymethyl)-24-hydroxy-5β-cholestan-26-oyl-4′-isopropyl-oxazolidin-2′-one** δ ^{1}H (400MHz, $CDCl_3$) 0.68 (3H, s, 18-Me), 0.89 (3H, s, 19-Me), 0.91 (3H, d, *J* = 7.0 Hz, H-7′), 0.94 (3H, d, *J* = 6.9 Hz, H-8′), 0.99-1.01 (1H, m, H-1), 1.04 (3H, d, *J* = 6.5 Hz, 21-Me), 1.10-1.14 (1H, m, H-15), 1.26 (3H, d, *J* = 6.9 Hz, 25-Me), 1.32-1.50 (9H, m, H-2, H-4, H-5, H-15, H-16, H-20, H-22, H-23), 1.52-1.70 (5H, m, H-2, H-6, H-8, H-11, H-17), 1.74-2.04 (7H,

m, H-1, H-6, H-9, H-11, H-14, H-16, H-22), 2.18-2.26 (2H, m, H-4), 2.31-2.38 (1H, m, H-6′), 3.40 (9H, s, OMe), 3.53-3.55 (1H, m, H-3), 3.56-3.59 (6H, m, H-3′), 3.63-3.65 (1H, m, H-7), 3.68-3.72 (6H, m, H-2′), 3.71-3.77 (1H, m, H-25), 3.80-3.85 (1H, m, H-12), 3.87-3.92 (1H, m, H-24), 4.19-4.27 (2H, m, H-5′), 4.43-4.51 (1H, m, H-4′), 4.76-4.84 (6H, m, H-1″); δ ^{13}C (100MHz, $CDCl_3$) 11.7 (25-Me), 12.9 (C-18), 17.1 (C-7′), 17.5 (C-8′), 18.1 (C-19), 23.8 (C-21), 23.4-32.7 (C-1, C-2, C-11, C-15, C-16, C-20, C-22, C-23, C-6′), 36.2-48.5 (C-4, C-5, C-6, C-8, C-9, C-10, C-13, C-14, C-17, C-25), 58.3 (C-4′), 59.4 (OMe), 65.8 (C-5′), 67.0 (C-2″), 71.0 (C-24), 72.2 (C-3″), 74.9 (C-3), 77.2 (C-7), 79.9 (C-12), 94.5 (C-1″), 152.7 (C-2′), 175.1 (C-26); HRMS calcd for $C_{45}H_{79}NO_{13}Na$ ($M+Na^+$) 864.5484; found 864.5449.

***N*-(24*S*,25*R*,4′*S*)-(3α,7α,12α)-*tris*(methoxyethoxymethyl)-24-fluoro-5β-cholestan-26-oyl-4′-isopropyl-oxazolidin-2′-one (18)** δ ^{1}H (400MHz, CDCl3) 0.68 (3H, s, 18-Me), 0.89 (3H, s, 19-Me), 0.91 (3H, d, *J* = 7.0 Hz, H-7′), 0.94 (3H, d, *J* = 6.9 Hz, H-8′), 0.99-1.01 (1H, m, H-1), 1.04 (3H, d, *J* = 6.5 Hz, 21-Me), 1.10-1.14 (1H, m, H-15), 1.26 (3H, d, *J* = 6.9 Hz, 25-Me), 1.32-1.50 (9H, m, H-2, H-4, H-5, H-15, H-16, H-20, H-22, H-23), 1.52-1.70 (5H, m, H-2, H-6, H-8, H-11, H-17), 1.74-2.04 (7H, m, H-1, H-6, H-9, H-11, H-14, H-16, H-22), 2.18-2.26 (2H, m, H-4), 2.31-2.36 (1H, m, H-6′), 3.40 (9H, s, OMe), 3.53-3.55 (1H, m, H-3), 3.56-3.59 (6H, m, H-3″), 3.63-3.65 (1H, m, H-7), 3.68-3.72 (6H, m, H-2″), 3.80-3.85 (1H, m, H-12), 4.10-4.18 (1H, m, H-25), 4.19-4.25 (2H, m, H-5′), 4.44-4.49 (1H, m, H-30), 4.69-4.82 (1H, m, H-24), 4.76-4.84 (6H, m, H-1″) δ ^{13}C (100MHz, $CDCl_3$) 11.7 (25-Me), 12.9 (C-18), 17.1 (C-7′), 17.5 (C-8′), 18.1 (C-19), 23.8 (C-21), 23.4-32.7 (C-1, C-2, C-11, C-15, C-16, C-20, C-22, C-23, C-6′), 36.2-48.5 (C-4, C-5, C-6, C-8, C-9, C-10, C-13, C-14, C-17), 42.8 (C-25, d, *J* = 19 Hz), 58.3 (OMe), 59.4 (OMe), 65.8 (C-5′), 67.0 (C-2″), 72.2 (C-3″), 74.9 (C-3), 77.2 (C-

7), 79.9 (C-12), 94.5 (C-1″), 95.2 (C-24, d, *J* = 155 Hz), 152.7 (C-2′), 175.1 (C-26); HRMS calcd for $C_{45}H_{78}FNO_{12}Na$ ($M+Na^+$) 866.5423; found 866.5406.

(24*S*,25*R*)-(3α,7α,12α)-*tris*(methoxyethoxymethyl)-24-fluoro-5β-cholestan-26-oic acid δ ^1H (400MHz, $CDCl_3$) 0.68 (3H, s, 18-Me), 0.89 (3H, s, 19-Me), 0.96 (3H, d, *J* = 6.5 Hz, 21-Me), 0.99-1.01 (1H, m, H-1), 1.13-1.18 (1H, m, H-15), 1.24 (3H, d, *J* = 6.9 Hz, 25-Me), 1.32-1.50 (9H, m, H-2, H-4, H-5, H-15, H-16, H-20, H-22, H-23), 1.52-1.70 (5H, m, H-2, H-6, H-8, H-11, H-17), 1.74-2.04 (7H, m, H-1, H-6, H-9, H-11, H-14, H-16, H-22), 2.22-2.30 (2H, m, H-4), 2.74-2.84 (1H, m, H-25), 3.40 (9H, s, OMe), 3.53-3.55 (1H, m, H-3), 3.56-3.59 (6H, m, H-3′), 3.63-3.65 (1H, m, H-7), 3.68-3.72 (6H, m, H-2′), 3.80-3.85 (1H, m, H-12), 4.53-4.69 (1H, m, $J_{H24\text{-}F}$ = 38.2 Hz, H-24), 4.76-4.84 (6H, m, H-1′); δ ^{13}C (100MHz, CDCl3) 11.7 (25-Me), 12.9 (C-18), 18.1 (C-19), 23.8 (C-21), 23.4-32.7 (C-1, C-2, C-11, C-15, C-16, C-20, C-22, C-23), 36.2-48.5 (C-4, C-5, C-6, C-8, C-9, C-10, C-13, C-14, C-17), 42.1 (C-25, d, *J* = 19 Hz), 59.4 (OMe), 67.0 (C-2′), 72.2 (C-3′), 74.9 (C-3), 77.2 (C-7), 79.9 (C-12), 94.5 (C-1′), 95.2 (C-24, d, *J* = 155 Hz), 175.2 (C-26); HRMS calcd for $C_{39}H_{69}FO_{11}Na$ ($M+Na^+$) 775.4708; found 755.4722.

(24S,25R)-(3α,7α,12α)-trihydroxy-24-fluoro-5β-cholestan-26-oic acid (19) δ ^1H (400MHz, $CDCl_3$) 0.74 (3H, s, 18-Me), 0.94 (3H, s, 19-Me), 0.99-1.01 (1H, m, H-1), 1.04 (3H, d, *J* = 6.5 Hz , 21-Me), 1.11-1.13 (1H, m, H-15), 1.18 (3H, d, *J* = 6.9 Hz, 25-Me), 1.32-1.50 (9H, m, H-2, H-4, H-5, H-15, H-16, H-20, H-22, H-23), 1.52-1.70 (5H, m, H-2, H-6, H-8, H-11, H-17), 1.74-2.04 (7H, m, H-1, H-6, H-9, H-11, H-14, H-16, H-22), 2.18-2.26 (2H, m, H-4), 2.67-2.81 (1H, m, H-25), 3.35-3.39 (1H, m, H-3), 3.80-3.84 (1H, m, H-7), 3.97 (1H, t, *J* = 2.5 Hz, H-12), 4.48-4.67 (1H, m, $J_{H24\text{-}F}$ = 41.6Hz, H-24); δ ^{13}C (100MHz, $CDCl_3$) 11.7 (25-Me), 12.9 (C-18), 18.1 (C-19), 23.8 (C-21), 23.4-32.7 (C-1, C-2, C-11, C-15, C-16, C-20, C-22, C-23), 36.2-48.5 (C-4, C-5, C-6, C-8, C-9, C-10, C-13,

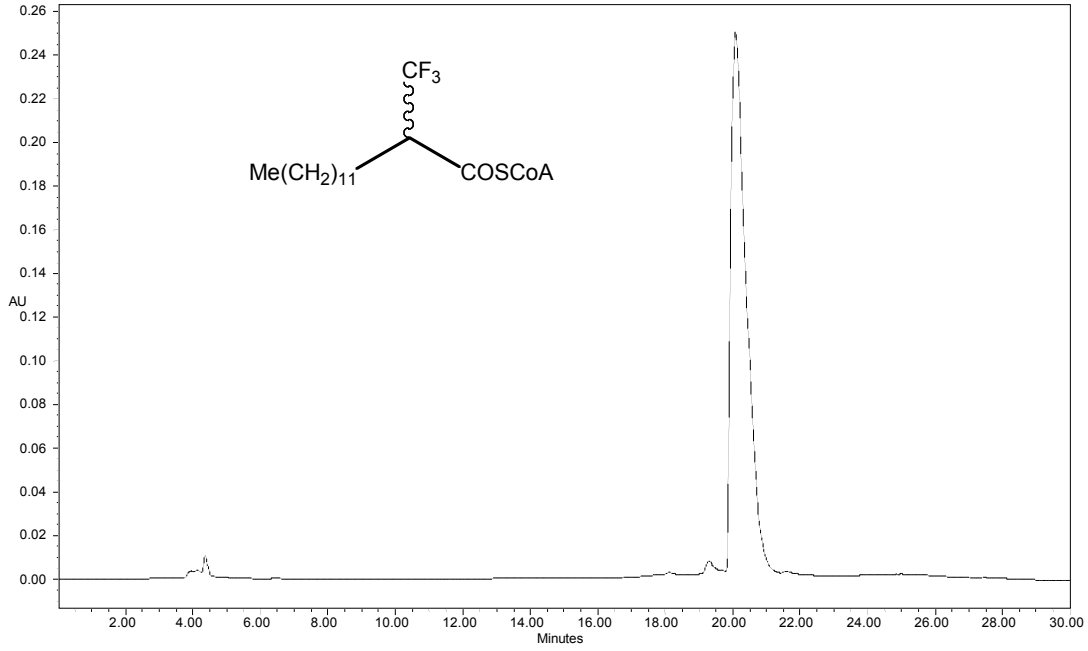
C-14, C-17), 42.1 (C-25, d, J = 19 Hz), 69.4 (C-7), 73.3 (C-3), 74.4 (C-12), 95.2 (C-24, d, J = 155 Hz), 175.2 (C-26); HRMS calcd for $C_{27}H_{44}FO_5$ ($M+H^-$) 467.3165; found 467.3173

2. HPLC traces of isolated CoA-thioesters

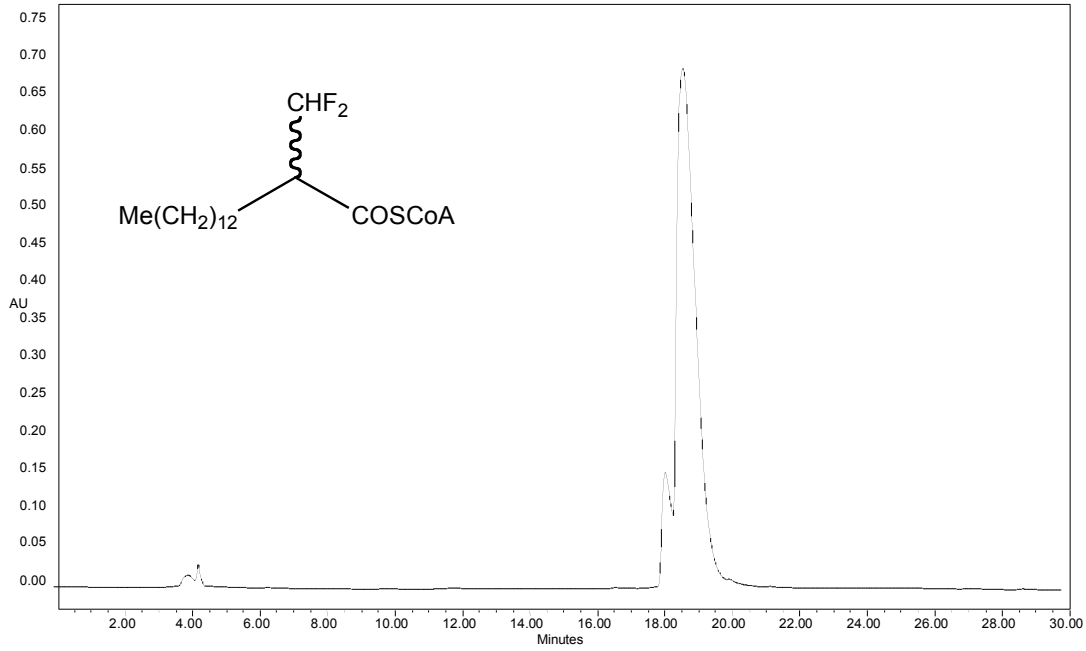
Method 1

The following analytical reverse phase HPLC was performed on a Waters 2695 separations module equipped with a Waters 996 photo diode array detector. Separations were carried out using an Alltech Altima C-18 5μ, 250mm x 4.6mm column packed with 5μm spherisorb ODS silica. A gradient using 90% MeOH/H_2O and 50% MeOH/25mM K_2HPO_4/KH_2PO_4 was used. Injection volume ranged from 20-80μL.

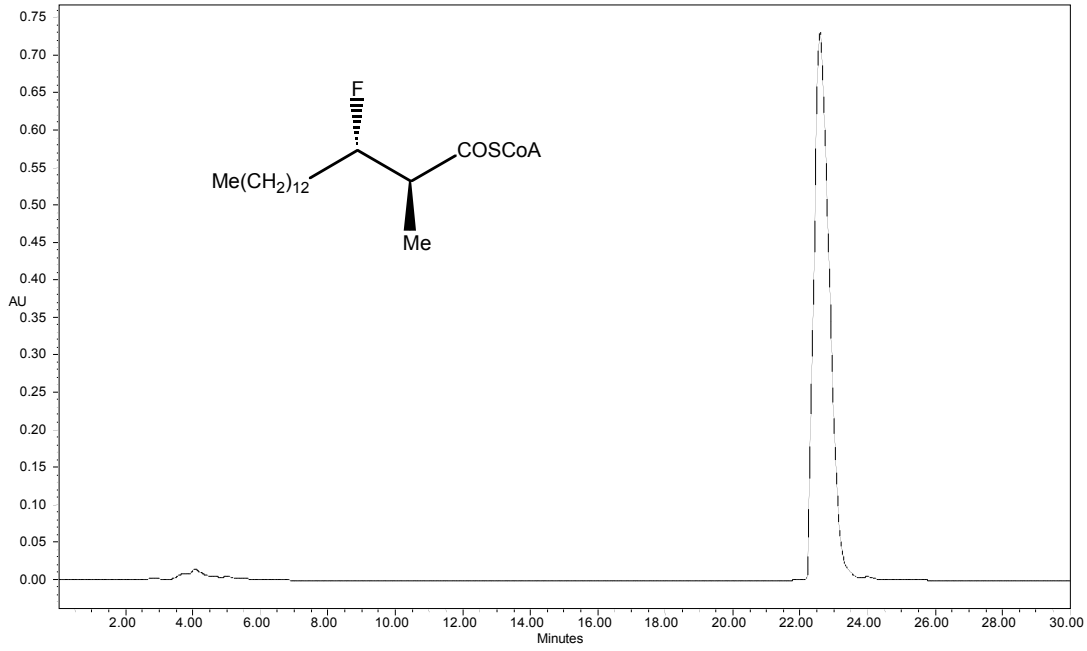
2-trifluoromethyltetradecanoyl-CoA **3**



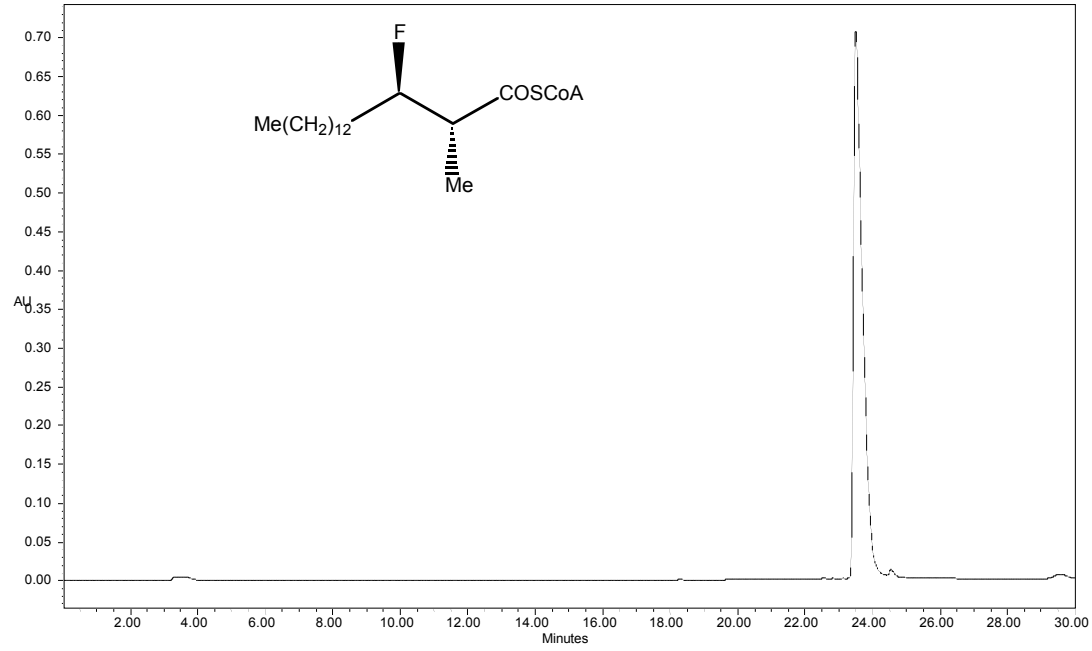
2-difluoromethylpentadecanoyl-CoA **4**

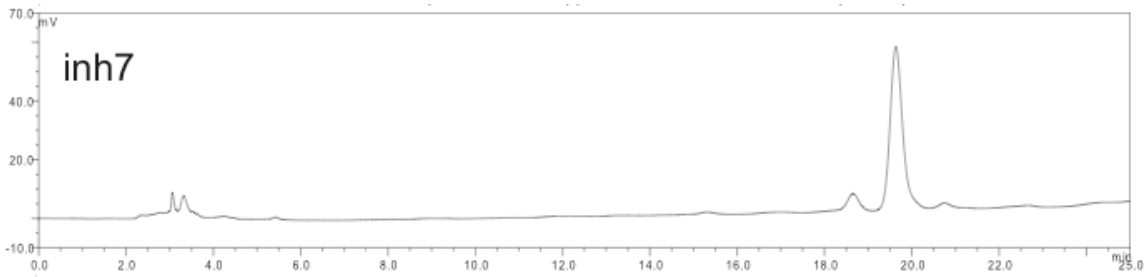


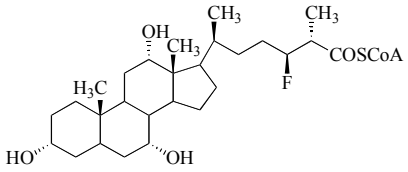
(2*R*,3*S*)-3-fluoro-2-methylhexadecanoyl-CoA **5**



(2*S*,3*R*)-3-fluoro-2-methylhexadecanoyl-CoA **6**



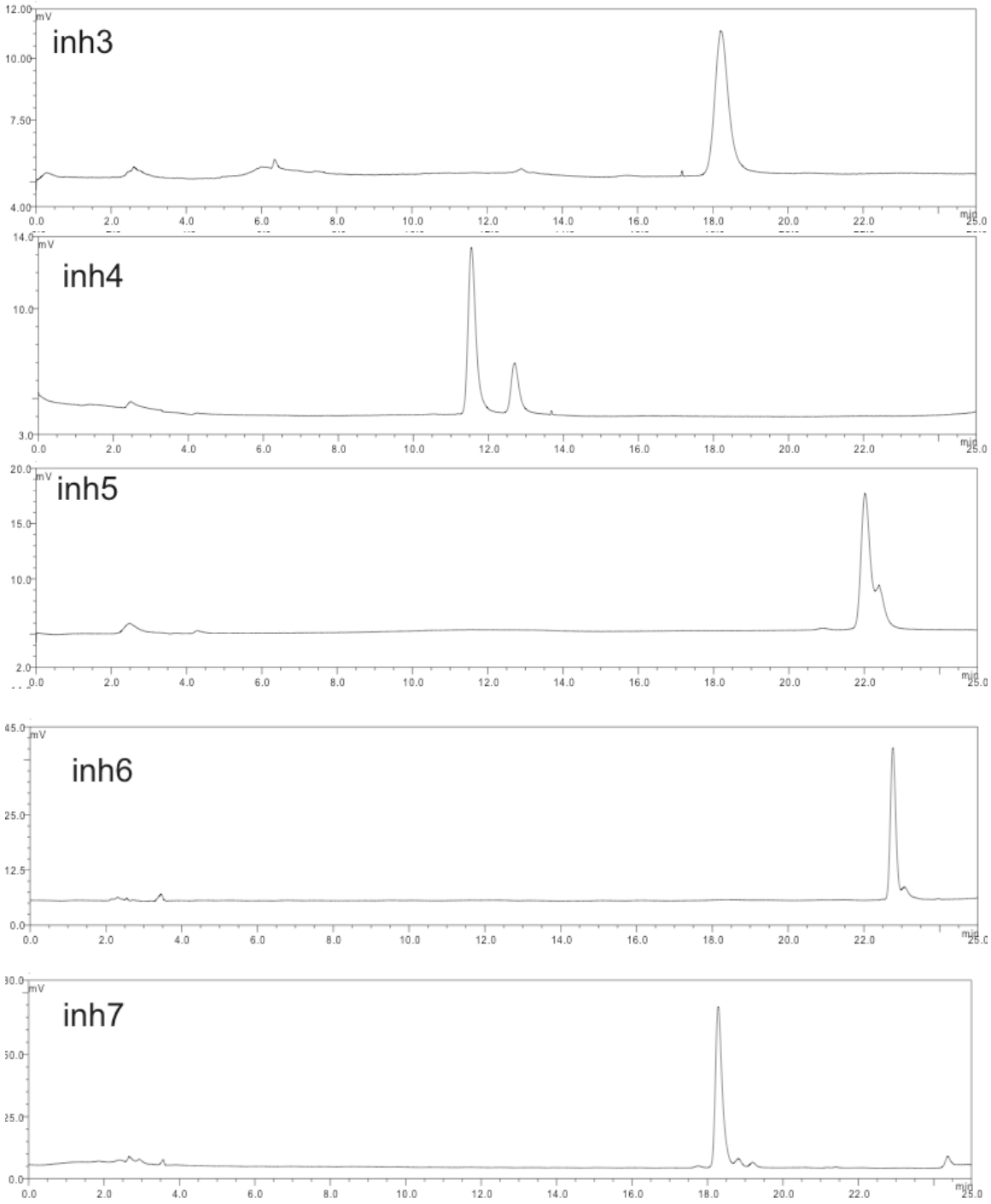




7

Method 2

The following reverse phase HPLC analyses were carried out using a Perkin Elmer Biocompatible Binary Pump 250 and a Pharmacia UV detector LKB-UV-MII. Separations were carried out using a SupelcoSIL LC-18-DB column 250 mm x 4.6 mm. The gradient used was 40 to 70% A:B; (A= 70% MeCN/16.9mM sodium phosphate pH 6.9, B = 10% MeCN/16.9mM sodium phosphate pH6.9).



HPLC analysis data for CoA esters 3-7.

	Method 1		Method 2	
Compound	Retention time (min)	% purity	Retention time (min)	% purity
3	20.4	98.9	18.2	98.8
4[a]	18.7	79.4	11.6	76.7
5[a]	23.0	>99	22.0	77.4
6	23.7	>99	22.7	92.9
7	18.3	92.7	19.6	89.5

[a]Compounds **4** and **5** are mixtures of diastereoisomers resulting from use of racemic acid and suspected epimerisation of the α-centre respectively. The minor diastereoisomer for **5** is only resolved by method 2. Retention times are quoted for the major diastereoisomer in each case.

3. AMACR Inhibition Assay

AMACR activity was determined by monitoring the interconversion of the (25*R*/*S*)-isomer of THC-CoA **2**. As AMACR source an organellar pellet prepared by centrifugation (16,000 x *g*) of a rat liver homogenized in 250 mM sucrose, 5 mM MOPS, 2 mM EDTA and 0.1% ethanol (final pH 7.4) was used. The incubations consisted of 0.005 mg/ml of rat liver protein, 100 mM MOPS pH 7.0 and 50 μM (25*S*)- or (25*R*)-THC-CoA without or with increasing concentrations of the different inhibitor compounds (0-200 μM dissolved in 20 mM MES, pH 6.0). Reactions were allowed to proceed for 20 minutes at 37°C and terminated by the addition of HCl (0.18 M), followed by resolution of (25*S*)- or (25*R*)-THC-CoA by HPLC as described (Nat Genet. 2000 Feb; 24 (2):188-91). HPLC was carried out with a reversed-phase C18-column (Alltima 250 mm x 4.6 mm, Alltech) and a linear gradient of methanol in potassium phosphate buffer (50 mM, pH 5.3). Kinetic parameters were determined using direct linear plot analysis.

4. Cytotoxicity Assay

PC3, CWR22Rv1, and Du145 cells were obtained from American Type Culture Collection and were grown in RPMI 1640 media supplemented with 10% fetal bovine serum (Life Technologies Inc.). PrEC cells (non-immortalized primary cultures of benign prostate epithelium) were obtained from Dr. John Isaacs at Johns Hopkins University and were maintained in supplemented PrEBM media (Cambrex Bioscience).

Individual T-75 flasks of each cell type were trypsinized and the cells were counted for viability and concentration using the Guava ViaCount Kit and Guava EasyCyte flow cytometer (Guava Technologies Inc.). 2,500 cells were plated, in triplicate, for each time point (Days 1- 10), one plate per day. After allowing the cells to attach overnight, the plating media was withdrawn and replaced with corresponding media containing desired compound concentrations (50uM or 100uM) or vehicle control (0.02% β-cyclodextrin, 0.04% β-cyclodextrin). Each plate also contained a media alone control in triplicate. The cells were then allowed to incubate in their respective media for the given time period (Day 1 corresponds to the first 24 hours in the presence of the respective compound) and the MTT assay was performed as directed by the manufacturer (Roche Applied Sciences). The data was then normalized as a percentage of vehicle control. A two-tailed Student's T-Test was performed for each triplicate day compared to its vehicle control. Any time point for which the p-value is less that 0.05 is marked with an asterisk(*).

5. Western Blot Analysis

Cell lysates of each respected cell type were prepared using RIPA buffer (Pierce Biotechnologies) and HALT© protease inhibitors (Pierce Biotechnologies). Thirty-five micrograms of each lysate was loaded, run on an SDS-PAGE gel (4%-15%, BioRad Inc.) and transferred electrophoretically to a nitrocellulose membrane (Amersham Biosciences). This membrane was blocked with 5% low fat dry milk and immunoblotted with a monoclonal antibody against AMACR (p504s, Zeta Corporation) in 5% low fat milk. The membrane was then washed and probed with an anti-mouse secondary antibody conjugated to horseradish peroxidase (HRP). Finally the membrane was washed and challenged with HRP chemiluminescent substrate (SuperSignal West Dura, Pierce Technologies) and exposed to film. This procedure was repeated, on an identical gel prepared in parallel containing the same samples, using a mouse monoclonal antibody direct at β-actin (Sigma Corp.) as a loading control and developed as described above.