SUPPLEMENT-- Synthesis of ADAM17 inhibitors

Three of the hydroxamate compounds synthesized for this study were new materials (NDH 4385, 4417, and 4409). The fourth, Abbot's ABT-518[1], was obtained by an alternative synthesis as described here.

Synthesis of NDH4409 (aka Olvanil hydroxamate or N-hydroxyolvanil or (9Z)-N-hydroxy-N-[(4-hydroxy-3-methoxyphenyl)methyl]-9-octadecenamide)

To oleoyl chloride (2.00 g, 6.65 mmol), 2-mercaptothiazoline (1.31 g, 6.33 mmol) was added with stirring. To this mixture, anhydrous dichloromethane (20 ml) and triethyl amine (0.88 ml, 6.33 mmol) were added. The reaction mixture was stirred overnight. Silica gel chromatography analysis (TLC) was used to assess the reaction. The solution was then diluted with dichloromethane and extracted with 1N hydrochloric acid (HCl). The two layers were allowed to separate, the aqueous layer was removed and the organic layer washed with saturated NaCl. The organic layer was treated with anhydrous magnesium sulfate, filtered and concerntrated to obtain the yellow oily crude product, which was purified by silica gel column chromatography using 5% ethyl acetate-hexane mixture as eluent. The yield of oleoyl thiazolide as a yellow oil was 87%: Rf = 0.38, CH_2Cl_2/hexanes, 4:6, v/v .

Oleoyl thiazolide (500 mg, 1.30 mmol) was added to a solution of reduced vanillin oxime (232 mg, 1.05 x 1.30 mmol) in dichloromethane. The resulting solution was stirred and triethyl amine (180 µl, 1.30 mmol) was added. Stirring continued overnight. The reaction mixture was diluted with dichloromethane and extracted with 3 x 50 ml of 1N HCl, then with saturated NaCl. The organic layer was first dried using anhydrous magnesium sulfate, then filtered and concentrated. The oily residue was purified with silica gel column chromatography using ethyl acetate-hexane, 3:7, v/v to give the product in a 66% yield. [1H NMR (CDCl3) δ 6.88-6.75 (m,

3H), 5.36-5.28 (m, 2H), 4.72 (s, 2H), 3.87 (s, 3H), 1.98 (d, J = 6.15 Hz, 4H), 1.64 (s, 2H), 1.54 (s, 2H), 1.27-1.24 (m, 22 H), 0.86 (t, J = 6.55 Hz, 3H).]

Synthesis of NDH4385 (aka Retro-OH-7; N-hydroxy-heptylhomovanillamide; N-heptyl-N-hydroxy-2-[4-hydroxy-3-methoxyphenyl] acetamide)

Step 1--Synthesis of N-(benzyloxy) heptan-1-imine: O-benzylhydroxylamine (369 mg, 349 µl, 3.0 mmol) was added to heptanal (343 mg, 420 µl, 3.0 mmol) and purged with N_2. The exothermic reaction yielded a cloudy solution which was stirred at room temperature for four min before adding 3 mL of dry tetrahydrofuran (THF). Stirring continued for 20 min, at which time thin layer chromatography (TLC) (with hexanes/ethyl acetate, 85:15) indicated completion of reaction. The volatiles were removed under reduced pressure, and the crude material was purified by silica gel column chromatography using hexanes/ethyl acetate (95:5) as eluent to give 599 mg (91% yield) of the imine as a clear oil. Rf = 0.48 and 0.51 (two isomers) (hexanes/ethyl acetate, 95:5, v/v); [1H NMR (CDCl3) δ 7.43 (t, 3J = 6.3 Hz, 0.6H), 7.36-7.31 (m, 4H), 7.30-7.26 (m, 1H), 6.66 (t, 3J = 5.3 Hz, 0.4H), 5.09 (s, 0.8H), 5.04 (s, 1.2H), 2.37-2.33 (m, 0.8H), 2.19-2.15 (m, 1.2H), 1.48-1.42 (m, 2H), 1.34-1.23 (m, 6H), and 0.86 (t, 3J = 7.0 Hz, 3H)].
Step 2-- Synthesis of N-(benzyloxy)heptylamine: The product prepared above, (570 mg, 2.60 mmol) was dissolved in 15 ml of high performance liquid chromatography (HPLC) grade methanol. To the stirred solution was added methyl orange indicator, followed by sodium cyanoborohydride ($NaCNBH_3$) (817 mg, 5 x 2.60 mmol). Dropwise addition of concentrated HCl, at intervals over a 2 hr time period, was carried out until the indicator's pink color persisted for 30 min. The mixture was stirred overnight at room temperature, and the next day the methanol was removed under reduced pressure in a fume hood. The dry residue was taken up in 100 ml of dichloromethane (CH_2Cl_2) and the resulting solution extracted with 75 ml of 1N

potassium hydroxide (KOH). The aqueous layer was collected and extracted with 2 x 40 mL of CH_2Cl_2. The organic extracts were combined and dried over $MgSO_4$, filtered and concentrated. Purification by silica gel column chromatography, eluting with CH_2Cl_2/hexanes (9:1), gave 475 mg (83%) of a clear oil. Rf = 0.36 (dichloromethane/hexanes, 95:5, v/v). [1H NMR (CDCl) δ 7.36-7.28 (m, 5H), 4.77 (s, 2H), 2.96-2.93 (m, 2H), 1.57-1.51 (m, 2H), 1.33-1.22 (m, 8H) and 0.86 t, 3J = 7.0 Hz, 3H). Exact mass (FAB+) calculated for $C_{14}H_{24}NO$ [M + H] 222.1852, found 222.1861.]

Step 3--Synthesis of N-benzyloxy-N-heptyl-2-(4-hydroxy-3-methoxyphenyl)acetamide:

To a flask containing 10 ml of dry CH_2Cl_2 were added homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid) (182 mg, 1.0 mmol), N-hydroxbenzotriazole (HOBt) (142 mg, 1.05 mmol), and N-benzyloxyheptylamine (prepared as in step 2, 232 mg, 1.05 mmol). 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (211 mg, 1.1 mmol) was added and stirring continued overnight. The reaction solution was diluted with CH_2Cl_2 and extracted with 1N HCl, saturated $NaHCO_3$ and saturated NaCl. The resulting organic layer was dried over magnesium sulfate ($MgSO_4$), filtered and concentrated. The residue was purified by silica gel column chromatography, eluting first with CH_2Cl_2, then with CH_2Cl_2/EtOH (98:2) and finally with CH_2Cl_2/MeOH (98:2) to give 358 mg (93%) of the title compound as a clear oil. Rf = 0.33 (dichloromethane/methanol, 98:2, v/v); [1H NMR (CDCl3) δ 7.38-7.33 (m, 5H), 6.80 (d, 3J = 8.0 Hz, 1H), 6.74 (bs, 1H), 6.68 (dd, 3J = 8.0 Hz, 4J = 1.5 Hz), 5.48 (bs, 1H, -OH), 4.74 (s, 2H), 3.78 (s, 3H), 3.62 (bs, 4H), 1.60-1.58 (m, 2H), 1.26-1.17 (m, 8H) and 0.84 (t, 3J = 7.0 Hz, 3H). Exact mass (FAB+) calculated for C23H32NO4 [M + H] 386.2356, found 386.2309.]

Step 4--Synthesis of the final product, N-heptyl-N-hydroxy-2-(4-hydroxy-3-methoxyphenyl) acetamide: To a flask containing palladium on carbon (5%), the solution of N-benzyloxy-N-heptyl-2-(4-hydroxy-3-methoxyphenyl) acetamide (358 mg, 0.93 mmol, prepared in Step 3

above) in 9.3 mL of dry MeOH was added with stirring at room temperature. The suspension was hydrogenolyzed at atmospheric pressure until uptake of H_2 was observed to have ceased (=7h). The suspension was filtered, and the filtrate was collected and concentrated. The crude product was purified by column chromatography on silica gel eluting with CH_2Cl_2/MeOH (98:2) to yield 216 mg (79%) of a brown solid (indicating a trace of an intensely color complex of iron). Rf = 0.13 (dichloromethane/methanol, 98:2, v/v). [1H NMR (CDCl3) δ 6.93-6.69 (m, 3H), 3.88 (bs, 3H), 3.80-3.40 (bs, 4H), 1.75-1.50 (bs, 2H), 1.243 (bs, 8H) and 0.86 bs, 3H). Exact mass (FAB+) calculated for $C_{16}H_{26}NO_4$ [M + H] 296.1856, found 296.1860.]

Synthesis of NDH4417 (aka, Retro-OH-8, N-hydroxy-octylhomovanillamide, or N-octyl-N-hydroxy-2-(4-hydroxy-3-methoxyphenyl) acetamide

NDH4417, a homologue of the product in the previous synthetic pathway, but with an additional carbon, was prepared by the same four step synthesis as NDH4385 as above. However, instead of starting with heptanal, NDH4417 synthesis commenced with octanal. The compound was obtained in 50% overall yield as a pale pink solid: Rf = 0.14 (dichloromethane/methanol, 98:2, v/v). [1H NMR (CDCl3) δ 6.83 (d, 3J = 8.0 Hz, 1H), 6.79 (bs, 1H), 6.68 (bd, 3J = 7.9 Hz, 1H), 3.86 (s, 3H), 3.62-3.55 (m, 3H), 3.46 (s, 1H), 1.63-1.53 (bm, 2H), 1.31-1.16 (m, 10H) and 0.86 (t, 3J = 6.9 Hz, 3H). Exact mass (FAB+) calculated for $C_{17}H_{28}NO_4$ [M + H] 310.2013, found 310.2025.]

Synthesis of NDH4450 (aka Abbott ABT-518 or N-[(1S)-1-((4S)-2,2-dimethyl-[1,3]dioxolan-4-yl)-2-(4-(4-(trifluoromethoxyphenoxy)phenylsulfonyl)ethyl]-N-hydroxyformamide)

The published method for this pharmaceutical[1] was followed as far as the synthesis of the penultimate intermediate, (4S)-4-((1S)-1-(hydroxyamino)-2-(4-(4-

trifluoromethoxyphenoxy)phenylsulfonyl) ethyl-2,2-dimethyl-[1,3]dioxolane, after which an alternative formylation transfer using N-formylmercaptothiazolide (2-thioxothiazolidine-3-carbaldehyde) was employed to yield ABT-518. (4S)-4-((1S)-1-(Hydroxyamino)-2-(4-(4-trifluoromethoxyphenoxy phenylsulfonyl)ethyl-2,2-dimethyl-[1,3]dioxolane (0.257 mmol) was dissolved in CH_2Cl_2 (5 mL). N-Formylmercaptothiazolide (1 equivalent), dissolved in CH_2Cl_2 (3 mL), was added dropwise to the reaction flask. Triethylamine (1 equivalent) was then added and the reaction contents were left stirring at ambient temperature for 2 hr, after which the yellow color of the thiazolide had disappeared. The crude product was purified by silica gel chromatography using hexanes and ethyl acetate (70:30) as the eluent, and yielded 86 mg (66%) of pure product (ABT-518) as a white semi-solid. Rf = 0.12 (hexanes:ethyl acetate, 3:2, v/v). [1H NMR (DMSO): δ 1.13-1.27 (m, 6H), 3.27-3.30 (m, 0.6H), 3.58-3.66 (m, 2.1 H), 3.90-4.06 (m, 3H), 4.51-4.53 (t, 0.4H), 7.17-7.24 (m, 4H), 7.42-7.45 (d, 2H, J = 8.90 Hz), 7.77 (s, 0.6H), 7.84-7.87 (d, 0.8H, J = 8.85 Hz), 7.87-7.90 (d, 1.2H, J = 8.85 Hz), 8.08 (s, 0.4H), 9.58 (s, 0.6H), 9.95 (s, 0.4H).]

Reference Cited:

1. Wada CK, Holms JH, Curtin ML, et al. Phenoxyphenyl sulfone N-formylhydroxylamines (retrohydroxamates) as potent, selective, orally bioavailable matrix metalloproteinase inhibitors. J Med Chem 2002;45:219-232.