Preparation of the Soluble Epoxide Inhibitor SWE101 and the DOCK1 inhibitor TBOPP

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The National Institute on Aging has made a significant commitment to improving the treatment of Alzheimer's disease through the funding of the TREAT-AD network (Target Enablement to Accelerate Therapy Development for AD, https://treatad.org/). One of the goals of TREAT-AD is to create chemical tools to explore under-studied and unexplored targets related to Alzheimer's disease. In support of this effort, we sought to utilize two recently reported probes, SWE101 (**5**)[1] and TBOPP (**8**).[2] Due to the cost-prohibitive expense of commercial sources, in-house synthesis was undertaken with procedures adapted from literature.[1–3]

SWE101 is reported to be an inhibitor of soluble epoxide hydrolase (sEH) selective for the *N*-terminal phosphate domain, which is known to play a role in cholesterol metabolism.[1,4] The association of hypercholesterolemia with Alzheimer's disease sparked our interest in this target.[5]

In accordance with the published literature protocols, the preparation of SWE101 (Figure 1) started with an S_N2 substitution at the α-bromo position of phenacyl bromide **1** to afford **2**. Refluxing **2** neat with excess acetamide and boron trifluoride diethyl etherate provided the 1,3-oxazole **3**. Bromination of the oxazole ring with NBS and subsequent Suzuki coupling gave SWE101 **5**. All products were purified using normal phase silica gel chromatography. Notably, we found that inclusion of an acetic acid eluent modifier 0.1–3% minimized tailing seen during purification.

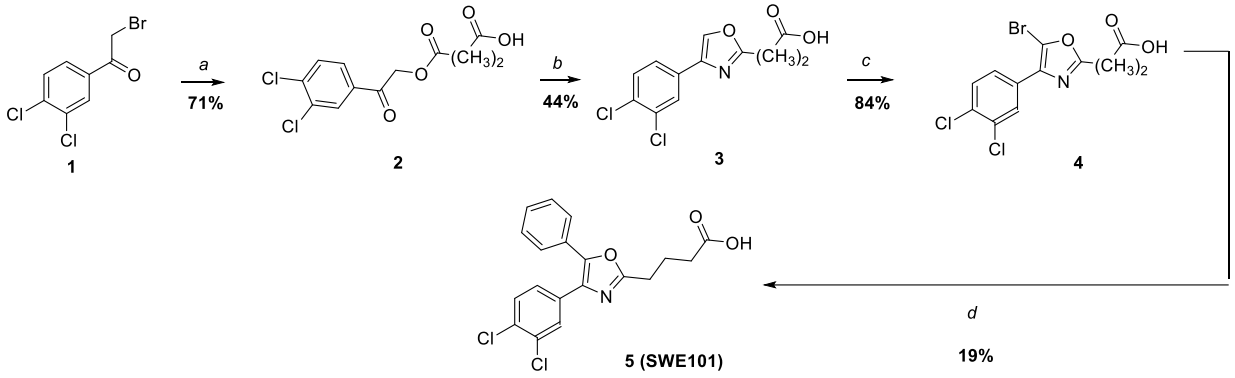


Figure 1. Preparation of SWE101 (**5**). *a)* **1** (2.0 mmol), glutaric acid (3 equiv.), Et_3N (3 equiv.), acetone (18 mL), 25 °C, 1.5 h. *b)* **2** (1.1 mmol), BF_3 * Et_2O (1.0 equiv), acetamide (4.5 equiv), neat, 140 °C, 20 h. *c*) **3** (0.67 mmol), *N*-Bromosuccinimide (1.05 equiv.), NH_4OAc (0.1 equiv.), CH_3CN (20 mL), 25 °C, 2 h . *d*) **4** (0.45 mmol), K_3PO_4 (3 equiv.), $Pd(PPh_3)_4$ (0.1 equiv.), $PhB(OH)_2$ (1.1 equiv.), 2:1 H_2O:DMF (6 mL), 80 °C, 48 h.

The reported inhibition of DOCK1-mediated Rac1 activation by TBOPP led to our desire to prepare this probe**.**[2] Rac1 inhibition is reported to decrease amyloid precursor protein (APP), which generates amyloid beta (Aβ) when improperly processed.[6] Additionally, Rac1 activation has also been found to influence tau hyperphosphorylation.[7]

The same synthetic scheme has been employed in both literature reports of TBOPP synthesis and was used by us as reported.[2,8] In our hands, the preparation afforded a pale brown solid (Figure 3), while the appearance was reported as a yellow oil in literature.[2]

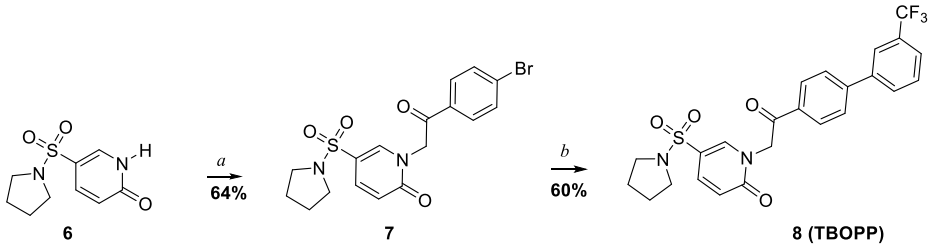
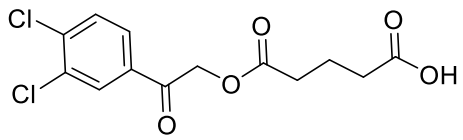
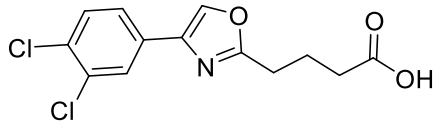


Figure 2. Preparation of TBOPP (**8**). *a*) **6** (0.50 mmol), 2-bromo-1-(4-bromophenyl)ethan-1-one (1.2 equiv.), NaH (1.1 equiv), THF (7.5 mL), 2.5 h. *b*) **7** (0.25 mmol), (3-(trifluoromethyl)phenyl)boronic acid (2 equiv.), K_3PO_4 (4 equiv.), $Pd(OAc)_2$ (0.1 equiv), $Pd(dppf)Cl_2$ (0.1 equiv.), 2.5:1 toluene:H_2O (3.5 mL), 100 °C, 8 h.

Experimental Procedure

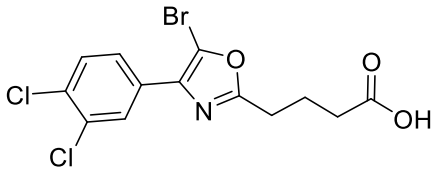


5-(2-(3,4-dichlorophenyl)-2-oxoethoxy)-5-oxopentanoic acid (**2**)*.* To a solution of glutaric acid (808.8 mg, 6.12 mmol) in acetone (40 mL) was added Et_3N (0.90 mL, 6.45 mmol) and the reaction stirred at room temperature for 30 min. Compound **1** (0.5402 g, 2.02 mmol) was added portionwise with stirring. The reaction was stirred with monitoring by TLC for disappearance of starting material (approx. 1 h). The reaction mixture was evaporated under vacuum and the residuepartitioned between aqueous HCl (10 mL, 1 N) and DCM (3 × 10 mL). The combined organic layers were evaporated to afford the crude product as a white solid (487 mg, 71% yield), which was used directly in the subsequent step. Further purification by silica gel chromatography (7:3 Hexanes:EtOAc w/ 0.1% AcOH) afforded the analytical sample. R_f = 0.20 (7:3 Hexanes:EtOAc w/ 0.1% AcOH). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.99 (d, *J* = 2.0 Hz, 1H), 7.74 (dd, *J* = 8.6, 2.0 Hz, 1H), 7.58 (d, *J* = 8.2 Hz, 1H), 5.28 (s, 2H), 2.57 (dt, *J* = 30.5, 7.4 Hz, 4H), 2.08 (quin, *J* = 7.2 Hz, 1H). ESI-MS: m/z = 319.4 $[M+H]^+$.

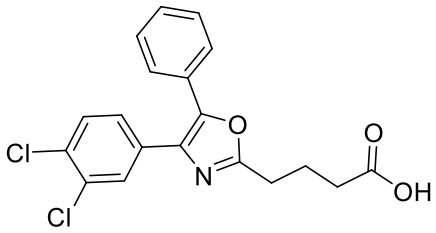


4-(4-(3,4-dichlorophenyl)oxazol-2-yl)butanoic acid (**3**): Compound **2** (381.3 mg, 2.2 mmol) and acetamide (620.3 mg , 10.5 mmol) were charged to a 50 mL round-bottom flask. The flask was fitted with a septum and purged with a nitrogen balloon. Boron trifluoride diethyl etherate (0.28 mL, 2.3 mmol) was added via syringe with stirring. The reaction was stirred neat for 20 h at 140 °C. The reaction mixture was poured into hydrochloric acid (1N, 25 mL), followed by extraction with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine, and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (4:1 Hexanes:acetone) to afford **3** as a light brown solid (214.3 mg, 44%); R_f = 0.23 (7:3 Hexanes:EtOAc w/ 0.1% AcOH),. ^{1}H

NMR (400 MHz, DMSO-d_6): δ = 8.64 (s, 1H), 7.74 (dd, J_1 = 8.6 Hz, J_2 = 2.0 Hz, 1H), 2.83 (t, J = 7.4 Hz, 2H), 2.34 (t, J = 7.2 Hz, 2H), 1.93 (quin, 7.4 Hz, 2H), ESI-MS: m/z = 300.0 $[M+H]^+$.

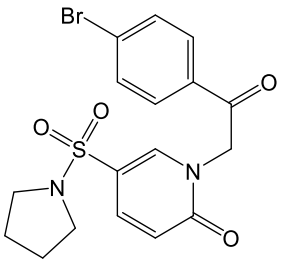


4-(5-bromo-4-(3,4-dichlorophenyl)oxazol-2-yl)butanoic acid (**4**): Ammonium acetate (5.1mg, 0.066 mmol) was added to a 4-dram vial w/ stir bar containing Compound **3** (201.7mg, 0.67 mmol) in 20 mL acetonitrile. *N*-bromosuccinimide (125.3g, 0.70 mmol) was added to the reaction mixture portions over 5 minutes. The reaction mixture was allowed to stir for 2 h. The reaction mixture was concentrated under pressure, and the residue was suspended in water (10 mL). The suspension was extracted with ethyl acetate (3 × 10 mL), and the organic layers evaporated under pressure to afford **5** the crude as a brown solid (214.1 mg, 84%) that was used directly in the next step. Further purification by silica gel chromatography (7:3 Hexanes:EtOAc w/ 1% AcOH) afforded the analytical sample. R_f = 0.29 (7:3 Hexanes:EtOAc w/ 0.1% AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.03 (d, J = 2.0 Hz, 1H), 7.86 (dd, J = 8.6 ,2.0 Hz, 1H), 7.77 (d, J = 8.6 Hz, 1H), 2.84 (t, J = 7.4 Hz, 2H), 2.35 (t, J = 7.2 Hz, 2H), 1.93 (quin, 7.4 Hz, 2H), ESI-MS: m/z = 378.0 and 380.3 ($[M+H]^+$, 1:1 ratio).



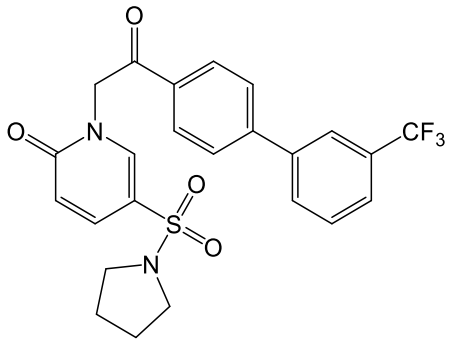
4-(4-(3,4-dichlorophenyl)-5-phenyloxazol-2-yl)butanoic acid (**5**): Tribasic potassium phosphate (289.4mg, 1.36 mmol) was weighed into a 4-dram vial and dissolved in water (4 mL) and DMF (2 mL). The vial was capped with a septum and the solution was degassed by bubbling with an argon balloon. Tetrakis(triphenylphosphine)-palladium(0) (57.4 mg, 0.05 mmol), Compound **4** (175.2 mg, 0.46 mmol), and phenylboronic acid (61.3 mg, 0.50 mmol) were added to the solution. After degassing again, the solution was stirred at 80 °C for 48 h under nitrogen. The reaction mixture was diluted with ethyl acetate (20 mL) and filtered through a plug of silica gel. After rinsing the silica gel with ethyl acetate, the filtrate was washed with aqueous HCl (1 N, 10 mL) and brine. The organic layer was dried with Na_2SO_4, concentrated under vacuum, and the residue was purified via silica gel chromatography (2:1 Hexanes:EtOAc +3% AcOH) to afford **5** (34.3 mg, 20%) as a pale yellow solid. R_f = 0.59 (7:3 Hexanes:EtOAc w/ 0.1% AcOH). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 7.77 (d, *J* = 2.0 Hz, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 7.47–7.58 (m, 6H), 2.89 (t, *J* = 7.0 Hz, 2H), 2.50 (t, *J* = 7.4 Hz, 2H), 1.99 (quin, 7.4 Hz, 2H), ESI-MS: m/z = 375.9 $[M+H]^+$.

Preparation of TBOPP:



1-(2-(4-bromophenyl)-2-oxoethyl)-5-(pyrrolidin-1-ylsulfonyl)pyridin-2(1H)-one (**7**): 5-(Pyrrolidin-1-ylsulfonyl)pyridin-2(1*H*)-one (**6**) (113.3 mg, 0.50 mmol) was dissolved in THF (5 mL), to which sodium hydride (13.1 mg, 0.55 mmol, 60% in mineral oil) was added at room temperature. A solution of 2-bromo-1-(4-bromophenyl)ethan-1-one (165.5 mg, 0.60 mmol) in THF (2.5 mL) was added to the slurry.

The resulting mixture was stirred for one h at 60 °C. After the reaction had cooled to room temperature, water (1 mL) was slowly added to quench the reaction. The reaction mixture was extracted with DCM (3 × 5 mL). The organic phase was washed with water (10 mL) then brine, and dried with Na_2SO_4. The solvent was removed under vacuum, and the residue purified via silica gel chromatography (1:1 DCM:EtOAc) to afford **7** (130.7 mg, 64%) as a pale yellow solid. R_f = 0.59 ^{1}H NMR (400 MHz, acetone-d_6): δ = 8.28 (d, *J* = 2.7 Hz, 1H), 8.07 (dt, *J* = 8.2, 2.4 Hz, 2H), 7.82 (dt, *J* = 8.6 Hz, 2.3 Hz, 2H), 7.78 (dd, *J* = 9.8 Hz, 2.7 Hz, 1H), 6.55 (d, *J* = 9.8 Hz, 1H), 5.69 (s, 2H), 3.26 (m, 4H), 1.86 (m, 4H) ppm. ESI-MS: m/z = 425.2 $[M+H]^+$.



5-(pyrrolidin-1-ylsulfonyl)-1-(3'-(trifluoromethyl)-[1,1'-biphenyl]-4-carbonyl)pyridin-2(1H)-one (**8**): Compound **7** (103.4 mg, 0.25 mmol), $Pd(OAc)_2$ (6.1 mg, 0.027 mmol), and $Pd(dppf)Cl_2$ (16.9 mg, 0.023 mmol) were slurried in toluene (2.5 mL). After stirring the mixture for five mins at 100 °C, degassed K_3PO_4 solution (4 M, 0.25 mL) was added to the mixture. After the solution had stirred an additional five mins at 100 °C, (3-(trifluoromethyl)phenyl)boronic acid (95.5 mg, 0.50 mmol) was added. After stirring for eight hours at 100 °C, the reaction mixture was extracted with DCM (3 × 5 mL), and the organic phase was washed with water (10 mL) then brine, and dried with Na_2SO_4. The solvent was evaporated under vacuum, and the residue was purified via silica gel chromatography using DCM:EtOAc (5:1) to afford **8** as a light brown solid (84.0 mg, 70%). R_f =

0.34 ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.13 (d, *J* = 8.2 Hz, 1H), 7.94 (d, *J* = 2.0 Hz, 1H), 7.89 (s, 1H), 7.83 (d, *J* = 7.4 Hz, 1H), 7.78 (d, *J* = 8.2 Hz, 2H), 7.70 (m, 2H), 7.64 (m, 1H), 6.67 (d, *J* = 9.8 Hz, 1H), 5.48 (s, 2H), 3.33 (m, 4H), 1.93 (m, 4H) ppm. ESI-MS: m/z = 491.3 $[M+H]^+$.

Acknowledgements

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