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Discovery of C-shaped aurone human neutrophil elastase inhibitors

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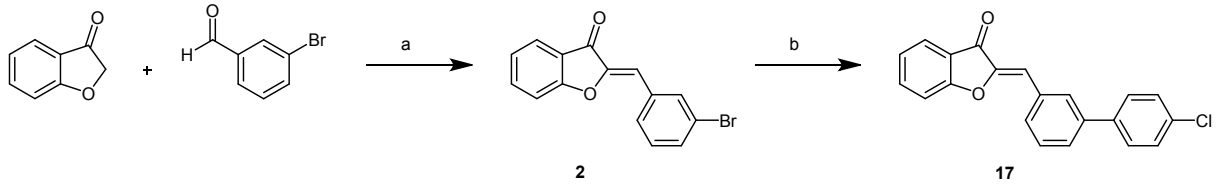
Chemistry

Chemical procedures for the synthesis of aurone compound in-house collection has been reported.[1] All tested compounds had a purity of ≥95% determined by elemental analysis. An example for compounds **2** and **17** is given bellow:



Scheme 1. Reagents and conditions: (a) Al_2O_3, MeOH, reflux under N_2, 24 hours; (b) (4-chlorophenyl)boronic acid, $Pd(PPh_3)_2Cl_2$, Na_2CO_3 (1M), 1,4-dioxane, 100 ^oC, 3 hours.

Synthesis of (*Z*)-2-(3-bromobenzylidene)benzofuran-3(2*H*)-one (2)

To a solution of benzofuran-3(2*H*)-one (134 mg, 1 mmol) in dry methanol (20 mL) at room temperature was added the 3-bromobenzaldehyde (1.2 mmol) and Al_2O_3 (1 mmol). The mixture was refluxed, under N_2, for 48 hours. After, the solvent was removed and the solid residue was dissolved in CH_2Cl_2. The organic layer was washed with water, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude product. The compound was purified by flash chromatography (Hexane/EtOAc = 95:5). Obtained as yellow solid, yield 54%, mp 123-124 ^oC. ^1H NMR (400 MHz, DMSO-d_6): δ = 8.18 (s, 1H), 8.03 (d, *J* = 7.9 Hz, 1H), 7.83-7.81 (m, 2H), 7.66 (d, *J* = 7.9 Hz, 1H), 7.61 (d, *J* = 8.2 Hz, 1H), 7.48 (t, *J* = 7.9 Hz, 1H), 7.34 (t, *J* = 8.2 Hz, 1H), 6.96 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 184.15, 166.02, 147.31, 138.42, 134.81, 133.88, 133.03, 131.56, 130.59, 124.90, 124.67, 122.68, 121.14, 113.82, 110.80. ESI-MS m/z (abund.): 625 $[M+M+Na]^+$ (100). Anal. Calcd. ($C_{15}H_9BrO_2$): C, 59.82; H, 3.02%. Found: C, 59.81; H, 3.05%.

Synthesis of (*Z*)-2-((4'-chlorobiphenyl-3-yl)methylene)benzofuran-3(2*H*)-one (17)

To a solution of the aurone **2** (0.23 mmol) in dioxane (2.3 mL) was added $Pd(PPh_3)_2Cl_2$ (0.023 mmol) and Na_2CO_3 1M (690 µL) followed by (4-chlorophenyl)boronic acid (0.28 mmol). The resulting mixture was degassed and stirred at 100 °C for 3 hours under N_2. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2, filtered under celite and concentrated under pressure to give the crude product. The compound was purified by flash chromatography (Hexane/EtOAc = 85:15). Obtained as yellow solid, 51% yield, mp 187-189 °C. 1H NMR (400 MHz, DMSO-d_6): δ = 8.24 (s, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.85-7.76 (m, 5H), 7.65-7.56 (m, 4H), 7.34 (t, *J* = 7.4 Hz), 7.06 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ = 183.82, 165.62, 146.64, 139.63, 138.33, 137.91, 132.83, 132.73, 130.35, 129.94, 129.89, 129.11, 128.67, 128.38, 124.47, 124.19, 120.88, 113.44, 112.11. Anal. Calcd. ($C_{21}H_{13}ClO_2$•0.15H_2O): C, 75.18; H, 4.00%. Found: C, 74.79; H, 4.20%.

Molecular Docking

3D structure coordinates of HNE were obtained from the Protein Data Bank, PDB code 3Q77 with X-ray coordinates at 2.00 Å resolution. To prepare the enzyme for the docking studies, the co-crystallized inhibitor as well as crystallographic waters included in the PDB structure, were removed. Hydrogen atoms were added and the protonation states were correctly assigned using the Protonate-3D tool within the Molecular Operating Environment (MOE) 2013.10 software package,[2] energy was minimized using MMFF94x forcefield. Molecular docking studies were then performed using the GoldScore scoring function from GOLD 5.2.0 software package[3] and each ligand was subjected to 2000 docking runs, HNE Val190 residue was defined as binding site as it

constitutes an important hydrophobic residue from the deep S1 pocket. The docking methodology was validated using the co-crystallised dihydropyrimidone inhibitor and is X-Ray pose was reproducible with RMSD of 0.5Å.

HNE biological assay

HNE activity was monitored at 25 °C for 30 min at excitation and emission wavelengths of 360 and 460 nm, respectively, in a microplate reader (FLUOstar Omega, BMG Labtech, Germany). For all compounds tested, the concentration of inhibitor that caused 50% inhibition of the enzymatic reaction (IC_{50}) was determined by non-linear regression using GraphPad PRISM software. Inhibitors stock solutions were prepared in DMSO, and serial dilutions were made in DMSO.

Fluorometric assay for HNE inhibition activity was carried out in 200 µL assay buffer (0.1 M HEPES pH 7.5 at 25 °C) containing 20 µL of 0.17 µM HNE (Merck, Germany) in assay buffer (stock solution 1.7 µM in 0.05 M acetate buffer, pH 5.5), 155 µL of assay buffer and 5 µL of each concentration of tested inhibitors. After 30 min of incubation at 25ºC the reaction was initiated by the addition of 20 µl of fluorogenic substrate to final concentration 200 µM (MeO-Suc-Ala-Ala-Pro-Val-AMC, Merck, Germany). The *K*m of this substrate of HNE was previously determined to be 185 µM (data not shown). Controls were performed using enzyme alone, substrate alone, enzyme with DMSO and a positive control (Sivelestat sodium salt hydrate, Sigma Aldrich, UK, determined IC_{50}=14nM).

Kinetic studies were performed for aurone **12**. The inhibitor and substrate were added to the enzyme with no previous incubation and product formation was monitored over 120 min, at different inhibitor concentrations.

References

1. M. P. Carrasco, A. S. Newton, L. Goncalves, A. Gois, M. Machado, J. Gut, F. Nogueira, T. Hanscheid, R. C. Guedes, D. J. dos Santos, P. J. Rosenthal and R. Moreira, *Eur J Med Chem*, 2014, **80**, 523-534.
2. MOE – Molecular Operating Environment MOE.2013.10 Chemical Computing Group: Montreal, www.chemcomp.com).
3. GOLD 5.2.0, CCDC Software Ltd; Cambridge, UK. (www.ccdc.cam.ac.uk/products/gold_suite).