SUPPLEMENTARY MATERIAL

Synthesis and biological evaluation of n-butylphthalide derivatives as anti-platelet aggregation agents

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Abstract

New analogues of n-butylphthalide (NBP) bearing various lengths of alkyl and different substitution at the 2-position of phthalide were designed and synthesized. Preliminary evaluation and prediction of ACD LogP software indicate that the derivatives display significantly improvement in water solubility than NBP does. Further biological analysis showed that NBP analogues specifically inhibit platelet aggregation induced by arachidonic acid (AA) but have no effect on that induced by

Adenosine 5-diphosphate (ADP). Especially compounds **1** and **3** were stronger than classical anti-platelet drug, aspirin, and equal potent with NBP, respectively. These findings provide an alternative approach to the development of NBP analogues with anti-platelet aggregation activity with good water solubility for the intervention of ischemic stroke.

Keywords n-butylphthalide analogues; Water solubility; Anti-platelet aggregation;

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Experimental

Materials

Adenosine 50-diphosphate (ADP) and Arachidonic Acid (AA), and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless stated other-wise. NBP derivatives were dissolved in dimethylsulfoxide (DMSO) and stored at -20°C .

Aqueous solubility

The solubilities of the compounds (50 mg) were de-termined in simulated intestinal fluid (pH 6.8) and simulated gastric fluid (pH 1.2). Briefly, the compounds were placed in an Eppendorf tubes containing 1 mL of the media, and were vortexed sufficiently at room tem-perature. Aliquots were centrifuged at 15,000 rpm for 10 min, and a 20 μL portion of the supernatant was analysed by HPLC. The solubility was calculated from a standard calibration curve of HPLC.

Lipophilicity (ClogP)

The clogP value was calculated using ACD LogP software package, version 4.55 (Advanced Chemistry Development Inc., Toronto, Canada).

Platelet aggregation studies

Blood was drawn from healthy volunteers, diluted with an equal volume of saline and kept stirring at 37°C in a platelet aggregometer (Verum Diagnostica, Munich, Germany). The anti-platelet effect was screened by Borns' method. Briefly, the samples were preincubated in duplicate for 5 min at 37°C with different concentra-tions (50 μM) of compound **1-6**, NBP, ASP or vehicle (0.1% DMSO) and the platelet aggregation was induced by Adenosine 5-diphosphate (ADP) and Arachidonic Acid (AA). The maximal aggregation was recorded light transmission within 5 min.

General

All solvents were commercially available and used without a further purification unless stated. The chemicals used were either purchased from commercial sources or prepared according to literature procedures. The ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance spectrometer 400 at 400 MHz and 100 MHz respectively. Chemical shifts are given in ppm (δ) referenced to CDCl_3 with 7.26 for ^1H and 77.10 for ^{13}C , and to d_6 -DMSO with 2.50 for ^1H and 39.50 for ^{13}C . In the case of multiplet, the signals are reported as intervals. Signals are abbreviated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Coupling constants are expressed in hertz. The progress of the reactions was monitored by thin-layer chromatography on a glass plate coated with silica gel with fluorescent indicator (GF254). Column chromatography was performed on silica gel (200-300 mesh). Purity of all final compounds was determined by Agilent 1100 HPLC system using an Eclipse XDB-C18 column. In HPLC conditions, flow rate was set at 1 mL/min, and gradient elution was run for 15 min, from 30% MeOH/ H_2O to 90% MeOH/ H_2O in 10 min and 90% MeOH/ H_2O in 5 min.

Synthesis

The synthetic routes of NBP analogues are outlined in Scheme 1 and Scheme 2. Generally, triethylamine (228 mg, 2.25 mmol), and 3-bromoisobenzofuran-1(3H)-one (400 mg, 1.88 mmol) was added to a stirred solution of aliphatic amines (2.25 mmol) in acetonitrile (8 mL). The reaction mixture was refluxed at 65 $^\circ\text{C}$ for several hours under argon atmosphere before acetonitrile was removed by rotary evaporation. The remained mixture was partitioned between saturated aqueous NaHCO_3 solution and EtOAc, and the aqueous phase was extracted with EtOAc. The combined organic phases were washed with H_2O and brine, dried over anhydrous Na_2SO_4 , evaporated in vacuo. The residue was purified by column chromatography on a silica gel

(DCM/EtOAc) to provide NBP derivatives **1-3**.

General procedure for the preparation of target compounds **4-6**: 15 mL of aliphatic amines was added to a flask containing 1.53 mmol of 2-cyanobenzaldehyde, and the solution was allowed to stir at 20 °C. After completion of the reaction, the redundant amines were removed by evaporation in vacuo. The obtained mixture was purified by column chromatograph on silica gel using CH₂Cl₂/MeOH as eluent to provide the desired compounds **5-6**. Compound **4** was obtained by dissolved in EtOAc and filtration.

Scheme 1 Synthesis of NBP analogues 1-3.

Scheme 2 Synthesis of NBP analogues 4-6.

3-(Prop-2-yn-1-ylamino)isobenzofuran-1(3H)-one (**1**). A white solid, 21.6% yield. HPLC t_R =5.273 min, 100%. ¹H NMR (400 MHz, CDCl₃) δ 7.74 (d, *J* = 7.5 Hz, 1H), 7.65 – 7.59 (m, 2H), 7.51 (t, *J* = 7.2 Hz, 1H), 5.99 (s, 1H), 4.54 (d, *J* = 17.7 Hz, 1H), 4.03 (dd, *J* = 17.6, 2.4 Hz, 1H), 2.24 (s, 1H) ppm. ¹³C NMR (100 MHz, CDCl₃) δ 167.1, 144.2, 132.5, 130.9, 129.5, 123.3, 123.0, 80.6, 77.8, 71.7, 28.2 ppm. IR ν 3280, 3048, 2917, 1689, 1436, 1347, 1273, 1212, 1046, 989, 944, 750, 697 cm⁻¹. LRMS (ESI, *m/z*): 188.1 [M + H]⁺. HRMS calcd for C₁₁H₈NO₂ [M-H]⁻: 186.0561, found 186.0657.

3-(Propylamino)isobenzofuran-1(3H)-one (**2**). A white solid, 50.4% yield. HPLC t_R = 6.621 min, 98.25%. ^1H NMR (400 MHz, CDCl_3) δ 7.61 – 7.42 (m, 4H), 5.74 (s, 1H), 3.51 – 3.44 (m, 1H), 3.30 – 3.23 (m, 1H), 1.68 – 1.59 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H) ppm. ^{13}C NMR (100 MHz, CDCl_3) δ 167.6, 144.0, 132.0, 131.4, 129.5, 123.2, 123.0, 81.6, 40.6, 21.4, 11.4 ppm. IR ν 3265, 2963, 2876, 1667, 1464, 1407, 1315, 1189, 1057, 879, 797, 745, 691, 588, 525 cm^{-1} . LRMS (ESI, m/z): 192.2 $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{11}\text{H}_{14}\text{NO}_2$ $[\text{M}+\text{H}]^+$: 192.1019, found: 192.0969.

3-(Butylamino)isobenzofuran-1(3H)-one (**3**). A yellow liquid, 36.3% yield. HPLC t_R = 11.268 min, 92.63%. ^1H NMR (400 MHz, CDCl_3) δ 7.61 – 7.53 (m, 3H), 7.43 (t, J = 7.4 Hz, 1H), 5.74 (s, 1H), 3.53 – 3.46 (m, 1H), 3.30 – 3.22 (m, 1H), 1.63 – 1.52 (m, 2H), 1.36 – 1.27 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H) ppm.

3-(Prop-2-yn-1-ylamino)isoindolin-1-one (**4**). A white solid, 47% yield. HPLC t_R = 4.861 min, 95.86%. ^1H NMR (400 MHz, CDCl_3) δ 7.83 (d, J = 6.8 Hz, 1H), 7.59 (s, 2H), 7.51 (s, 1H), 6.89 (s, 1H), 5.53 (s, 1H), 3.55 (dd, J = 46.8, 17.6 Hz, 2H), 2.33 (s, 1H), 1.82 (s, 1H) ppm. ^{13}C NMR (100 MHz, DMSO) δ 168.8, 145.7, 132.7, 131.7, 128.9, 123.9, 122.5, 82.6, 73.9, 69.0, 33.6 ppm. IR ν 3276, 3175, 3062, 2879, 1697, 1479, 1362, 1298, 1141, 1083, 1016, 832, 740, 590 cm^{-1} . LRMS (ESI, m/z): 187.1 $[\text{M}+\text{H}]^+$. HRMS calcd for $\text{C}_{11}\text{H}_{10}\text{N}_2\text{ONa}$ $[\text{M}+\text{Na}]^+$: 209.0685, found: 209.0609.

3-(Propylamino)isoindolin-1-one (**5**). A white flocculent solid, 93 % yield. HPLC t_R = 6.827 min, 98.99 %. ^1H NMR (400 MHz, CDCl_3) δ 7.82 (d, J = 7.6 Hz, 1H), 7.59 – 7.58 (m, 2H), 7.52 – 7.50 (m, 1H), 6.90 (s, 1H), 5.49 (s, 1H), 2.69 – 2.63 (m, 1H), 2.55 – 2.49 (m, 1H), 1.55 – 1.44 (m, 2H), 0.91 (t, J = 7.4 Hz, 3H) ppm.

3-(Butylamino)isoindolin-1-one (**6**). A white flocculent solid, 100% yield. HPLC t_R = 8.211 min, 96.19 %. ^1H NMR (400 MHz, CDCl_3) δ 7.82 (dt, J = 7.6, 1.0 Hz, 1H), 7.59 – 7.57 (m, 2H), 7.52 – 7.47 (m, 1H), 6.98 (s, 1H), 5.48 (s, 1H), 2.72 – 2.66 (m, 1H), 2.58 – 2.52 (m, 1H), 1.50 – 1.42 (m, 2H), 1.38 – 1.31 (m, 2H), 0.90 (t, J = 11.5

Hz, 3H) ppm.

^1H NMR ^{13}C NMR spectra