Arch. Pharm. Res.
DOI 10.1007/s12272-016-0737-5

Archives of Pharmacal Research
www.springer.com/12272

RESEARCH ARTICLE

Design and synthesis of 2,3-dihydro- and 5-chloro-2,3-dihydro-naphtho-[1,2-*b*]furan-2-carboxylic acid *N*-(substitutedphenyl)amide analogs and their biological activities as inhibitors of NF-κB activity and anticancer agents

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Received: 29 February 2016 / Accepted: 14 March 2016

Abstract A series of 2,3-dihydro- and 5-chloro-2,3-dihydro-naphtho-[1,2-*b*]furan-2-carboxylic acid *N*-(substitutedphenyl)amide analogs (**1a–k** and **2a–i**) were designed and synthesized for developing novel naphthofuran scaffolds as anticancer agents and inhibitors of NF-κB activity. Compound **1d**, which had a 4′-chloro group on the *N*-phenyl ring, exhibited inhibitory activity of NF-κB. Compound **2g**, which had a 5′-chloro group on the naphthofuran ring and a 3′,5′-bistrifluoromethane group on the *N*-phenyl ring, had the best NF-κB inhibitory activity. In addition, the novel analogs exhibited potent cytotoxicity at low concentrations against HCT-116, NCI-H23, and PC-3 cell lines. The two electron-withdrawing groups, especially at the 3′,5′-position on the *N*-phenyl ring, increased anticancer activity and NF-κB inhibitory activity. However, only 5-chloro-2,3-dihydronaphtho[1,2-*b*]furan-2-carboxylic *N*-(3′,5′-bis(trifluoromethyl)phenyl)amide (**2g**) exhibited both outstanding cytotoxicity and NF-κB inhibitory activities. This novel lead scaffold may be helpful for investigation of new anticancer agents by inactivation of NF-κB.

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Keywords Anticancer activity · Inhibition of NF-κB transcriptional activity · 2,3-Dihydronaphtho-[1,2-*b*]furan scaffolds

Introduction

Cancer is a large group of diseases that represent out-of-control proliferation of a particular cell type caused by oncogenes such as mutated genes, which allow unregulated cell growth (Campisi 2001). Oncogenes generate a large number of proteins involved in cancer cell signaling, which is triggered by the exposure to carcinogens such as chemicals, viruses, ultraviolet light, and radicals (Levy 1966; McCann et al. 1975; Sun 1990; Brash et al. 1991). In addition, oncogene up-regulation can lead to uncontrolled tumor growth through aberrant activation of cell signaling pathways by hormones, cytokines, and chemokines (Mallette et al. 2007; Mantovani et al. 2008; Borsig et al. 2014). Oncogenes are also activated by various transcription factors such as nuclear factor-κB (NF-κB) and signal transducer and activator of transcription (STAT) family proteins (Silva 2004; Gilmore 2006; Grivennikov and Karin 2009). NF-κB, an inducible transcription factor, is present in an inactive form in the cytoplasm in most cell types. Upon activation by external stimuli or abnormal signaling, NF-κB translocates to the nucleus and binds DNA or oncogenes. NF-κB binding regulates the expression of genes related to inflammation and unregulated cell growth (Gilmore 2006). Therefore, transcription factors such as NF-κB are regarded as key regulators of tumorigenesis. STAT3 is also one of transcription factors increasing the expression of proliferative and survival genes in cancers (Grivennikov and Karin 2009).

The STAT family, which consists of 7 proteins (STAT1–STAT4, STAT5A, STAT5B, and STAT6) in humans, is

Published online: 28 March 2016

considered to be important for cell survival and proliferation (Yu and Jove 2004). STAT3 regulates cell proliferation, cell cycle progression, apoptosis, angiogenesis, and immune evasion and influences cancer stem cells (Xiong et al. 2014). In interleukin-6 (IL-6)–stimulated hepatocytes, STAT3 acts as a DNA-binding factor mediated by the IL-6 family of cytokines and growth factors including epidermal growth factor (EGF) and hepatocyte growth factor (HGF) (Zhang et al. 2002; Aggarwal et al. 2009; Rebe et al. 2013). In breast, colon, gastric, lung, head and neck, skin, and prostate tumor cells, STAT3 is activated through phosphorylation of Janus kinase (JAK), which subsequently phosphorylates STAT3 (Siddiquee and Turkson 2008). Aberrant STAT3 activation in cancer cells increases resistance to apoptosis and chemotherapies aimed at initiating apoptosis (Mali 2015). Therefore, to suppress the proliferation of human cancer cells, various studies suggested agents that regulate oncogenes through inactivation of transcription factors such as NF-κB and STAT3 (Darnell 2002).

As part of our continuous efforts to design and develop novel scaffolds with anticancer activity and NF-κB inhibitory activity, we recently reported a series of novel benzofuran- and 2,3-dihydrobenzofuran-2-carboxamide analogs and explored their NF-κB inhibitory activities and cytotoxicity against various human cancer cell lines (Choi et al. 2015). Moreover, we reported that 5-chloro-2,3-dihydronaphtho [1,2-*b*]furan-2-carboxylic *N*-(3′,5′-bis(trifluoromethyl) phenyl)amide (**2g**) significantly inhibits liver tumor growth through activation of hepatocyte nuclear factor 4α (HNF4α) and inhibition of STAT3 (Kwon et al. 2015).

In our previous studies, chroman-, indoline- and dihydrobenzofuran-2-carboxamide analogs were designed and synthesized (Fig. 1a), and their NF-κB inhibitory activity and anticancer effects were evaluated (Kwak et al. 2007, 2008, 2009a, b, 2010; Choi et al. 2015). Among these analogs, KL-1156, the first hit compound, was reported as an inhibitor of NF-κB translocation to the nucleus in LPS-stimulated RAW 264.7 macrophage cells (Kim et al. 2004). Recently, it was confirmed that benzofuran- and 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(substitutedphenyl)amide analogs inhibited NF-κB in LPS-stimulated RAW 264.7 cells and had anticancer effects against six human cancer cell lines: ACHN (renal), HCT15 (colon), MM231 (breast), NUGC-3 (gastric), NCI-H23 (lung), and PC-3 (prostate). To obtain compounds with potent NF-κB inhibitory and anticancer activities, we designed novel scaffolds on the basis of them (Choi et al. 2015).

In many studies, various compounds containing the dihydronaphthofuran moiety as a core structure exhibited anti-proliferation effects against human cancer cell lines. For instance, 2-nitronaphthofuran and its analogs exhibit anticancer effects against hepatoma cells by activating HNF4α (Fig. 1b) (Le Guevel et al. 2009). 3-(3-Naphthofuranyl)-coumarin analogs and CX4152 also have anticancer activity (Chougala et al. 2015; Ha et al. 2015). Therefore, in this study we have designed and synthesized novel 2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxamide analogs to identify those with most potent anticancer and NF-κB inhibitory activities (Fig. 1c).

Materials and methods

Chemistry

All commercial chemicals were used as obtained and all solvents were purified prior to use by applying standard procedures. Air and moisture sensitive reactions were performed under an nitrogen atmosphere. All the products obtained were purified by column chromatography using silica gel (100–200 mesh). Thin layer chromatography was performed on E Merck silica gel GF-254 pre-coated plates; identification was performed under UV illumination. Flash column chromatography was performed on E Merck silica gel (230–400 mesh). Hexane was used as a co-eluent. ^{1}H and ^{13}C NMR were recorded in Bruker 400, 500 and 100, 125 MHz spectrometer respectively. The chemical shifts are reported in ppm downfield to TMS ($\delta = 0$) for ^{1}H NMR and relative to the central $CDCl_3$ resonance ($\delta = 77.0$) for ^{13}C NMR. IR spectra recorded on JASCO FT/IR-4100. High resolution mass spectra (HRMS) was obtained by using Agilent 1290 Infinity HPLC system and Agilent 6530 Q-TOF LC/MS system. Melting points were recorded on an Electro thermal IA 9100 apparatus and are uncorrected.

1-(Allyloxy)naphthalene

A mixture of 1-naphthol (**3**) (300 mg, 2.08 mmol), K_2CO_3 (575 mg, 4.16 mmol) and acetone (6.00 mL) was stirred for 30 min, then allyl bromide (0.216 mL, 2.50 mmol) was added at room temperature. The mixture was stirred for 2 h at 50–60 °C. After 2 h, the solvent was evaporated. Purification via flash column chromatography (EtOAc/hexanes = 1:9) provided 1-(allyloxy)naphthalene (98.4 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.32 (m, 1H), 7.80 (m, 1H), 7.50–7.46 (m, 2H), 7.43 (d, 1H, $J = 7.8$ Hz), 7.36 (t, 1H, $J = 7.8$ Hz), 6.82 (d, 1H, $J = 7.8$ Hz), 6.19 (tdd, 1H, $J = 17.3$, 10.9, 5.2 Hz), 5.55 (dd, 1H, $J = 17.3$, 1.6 Hz), 5.35 (dd, 1H, $J = 10.5$, 1.6 Hz), 4.73 (dd, 2H, $J = 5.2$, 1.6 Hz).

*2-Allylnaphthalen-1-ol (**4**)*

A mixture of 1-(allyloxy)naphthalene (205 mg, 1.11 mmol) and xylene (1.00 mL) was stirred for 16 h. After stirring for 16 h, the solvent was evaporated then purification via flash column chromatography (EtOAc/hexanes = 1:9) provided

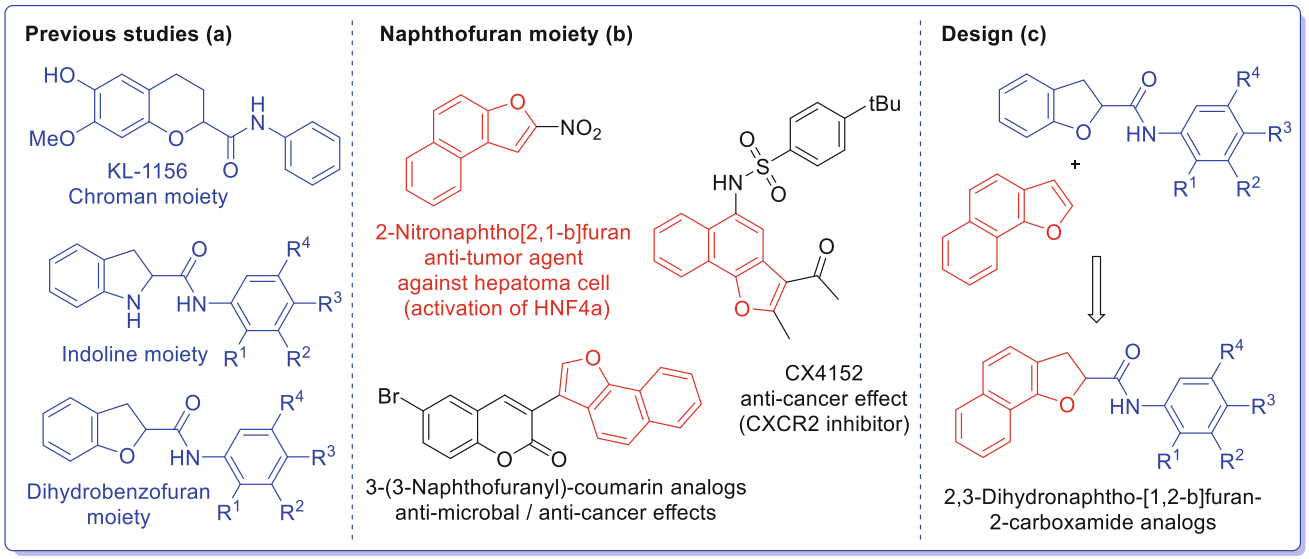


Fig. 1 **a** Structures of chroman, indoline, and 2,3-dihydrobenzofuran. **b** Biologically active scaffolds of naphthobenzofuran. **c** Design of 2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxamide analogs

2-allylnaphthalen-1-ol (87.6 %). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.17 (m, 1H), 7.78 (m, 1H), 7.48–7.44 (m, 2H), 7.41 (d, 1H, J = 8.4 Hz), 7.22 (d, 1H, J = 8.4 Hz), 6.09 (tdd, 1H, J = 17.1, 10.2, 6.2 Hz), 5.26 (dd, 1H, J = 17.1, 1.6 Hz), 5.24 (dd, 1H, J = 10.2, 1.6 Hz), 3.58 (dd, 2H, J = 6.2, 1.6 Hz).

2-Allyl-1-(benzyloxy)naphthalene

A mixture of 2-allylnaphthalen-1-ol (**4**) (436 mg, 2.37 mmol), K_2CO_3 (655 mg, 4.74 mmol) and acetone (50.0 mL) was stirred for 30 min. Then benzyl bromide (0.582 mL, 2.67 mmol) was added at room temperature. The mixture was stirred for 8 h at 50–60 °C. After stirring for 8 h, the solvent was evaporated and the reaction mixture was diluted by DCM. The mixture was washed with H_2O (3 × 20 mL), dried over anhydrous $MgSO_4$. Purification via flash column chromatography (EtOAc/hexanes = 1:9) provided 2-allyl-1-(benzyloxy)naphthalene (98.7 %). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.19 (d, 1H, J = 8.4 Hz), 7.88 (d, 1H, J = 8.4 Hz), 7.66 (d, 1H, J = 8.4 Hz), 7.61 (d, 2H, J = 8.4 Hz), 7.55–7.48 (m, 4H), 7.43 (t, 1H, J = 8.4 Hz), 7.39 (d, 1H, J = 8.4 Hz), 6.07 (tdd, 1H, J = 17.7, 9.4, 6.4 Hz), 5.15 (dd, 1H, J = 17.7, 1.6 Hz), 5.15 (dd, 1H, J = 9.4, 1.6 Hz), 5.08 (s, 2H), 3.66 (dd, 2H, J = 6.4, 1.6 Hz).

*2-((1-(Benzyloxy)naphthalen-2-yl)methyl)oxirane (**5**)*

A mixture of 2-allyl-1-(benzyloxy)naphthalene (455 mg, 1.66 mmol), *m*-CPBA (891 mg, 3.97 mmol) and dichloromethane (20.0 mL) was stirred for 6 h at 50–60 °C. After stirring for 6 h, the reaction mixture was diluted by DCM then washed with H_2O (3 × 20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$. Purification via flash column chromatography (EtOAc/hexanes = 1:8) provided 2-((1-(benzyloxy)naphthalen-2-yl)methyl)oxirane (72.9 %). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.14 (d, 1H, J = 8.5 Hz), 7.86 (d, 1H, J = 8.5 Hz), 7.64 (d, 1H, J = 8.5 Hz), 7.55 (d, 2H, J = 7.4 Hz), 7.52–7.43 (m, 5H), 7.39 (t, 1H, J = 7.4 Hz), 5.07 (s, 2H), 3.21 (m, 1H), 3.10 (dd, 1H, J = 14.4, 5.4 Hz), 3.04 (dd, 1H, J = 14.4, 5.4 Hz), 2.79 (dd, 1H, J = 5.1, 4.3 Hz), 2.59 (dd, 1H, J = 5.1, 2.5 Hz).

*(2,3-Dihydronaphtho[1,2-b]furan-2-yl)methanol (**6**)*

A mixture of 2-((1-(benzyloxy)naphthalen-2-yl)methyl)oxirane (**5**) (631 mg, 2.17 mmol), 10 % Pd/C (39.5 mg), K_2CO_3 (8.98 mg, 0.065 mmol), Et_3N (catalytic amount) and MeOH (20.0 mL) was stirred for 8 h at room temperature. After stirring for 8 h, the reaction mixture was filtered by Celite then solvent was evaporated. Purification via flash column chromatography (EtOAc/hexanes = 1:3) provided (2,3-dihydronaphtho[1,2-b]furan-2-yl)methanol (93.7 %). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.96 (d, 1H, J = 8.3 Hz), 7.81 (d, 1H, J = 8.3 Hz), 7.46–7.41 (m, 2H), 7.39 (d, 1H, J = 8.3 Hz), 7.32 (d, 1H, J = 8.3 Hz), 5.14 (m, 1H), 3.95 (dd, 1H, J = 12.3, 3.2 Hz), 3.84 (dd, 1H, J = 12.3, 6.3 Hz), 3.44 (dd, 1H, J = 15.4, 9.7 Hz), 3.20 (dd, 1H, J = 15.4, 7.6 Hz).

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid (**7**)*

To a stirred solution of (2,3-dihydronaphtho[1,2-b]furan-2-yl)methanol (**6**) (194 mg, 0.970 mmol) in CH_3CN/H_2O (1/

1, 2 mL) was added TEMPO (30.3 mg, 0.194 mmol), BAIB (687 mg, 2.13 mmol). After stirring for 5–6 h, the mixture was basicfied with sat. $NaHCO_3$ to pH 10–11 and washed with diethyl ether (10 ml × 3). The combined extracts were acidified with 1 *N* HCl to pH 2–3 then the mixture was extracted with EtOAc (3 × 20 mL). The combined extracts were dried over anhydrous Na_2SO_4. After evaporation, the product was dried in vacuo to afford the desired 2,3-dihydronaphtho[1,2-*b*]furan-2-carboxylic acid (65.8 %) as a brown oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.01 (d, 1H, J = 8.2 Hz), 7.83 (d, 1H, J = 8.2 Hz), 7.50-7.44 (m, 3H), 7.32 (d, 1H, J = 8.2 Hz), 5.46 (dd, 1H, J = 11.0, 6.6 Hz), 3.81 (dd, 1H, J = 15.7, 11.1 Hz), 3.62 (dd, 1H, J = 15.7, 6.6 Hz).

*General procedure for the synthesis of 2,3-dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(substitutedphenyl)amide analogs (**1a–1b**, **1d–g** and **1i**)*

To a mixture of acid (1.0 eq) and 1,1′-carbodiimidazole (1.2 eq) in anhydrous THF was stirred for 1 h then substituted aniline (1.0 eq) was added at room temperature. After stirring for 12–16 h, the reaction mixture was washed with H_2O (3 × 10 mL) then acidified with 1 *N*-HCl to pH 2. The mixture was extracted with EtOAc (3 × 10 mL) and the combined extracts were dried over anhydrous Na_2SO_4. After evaporation, the residue was purified by column chromatography (EtOAc/hexanes = 1:3–1:10).

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid N-phenylamide (**1a**)* Yield 30.6 %; m. p. 144.1–149.4 °C; IR 3057, 1683, 751 cm^{-1}; LRMS (ESI) m/z 312.2 (M + Na$^+$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.06 (d, 1H, J = 8.2 Hz), 7.87 (d, 1H, J = 8.2 Hz), 7.56–7.54 (m, 3H), 7.51–7.49 (m, 2H), 7.37–7.30 (m, 3H), 7.12 (t, 1H, J = 7.5 Hz), 5.48 (dd, 1H, J = 10.9, 6.5 Hz), 3.86 (dd, 1H, J = 16.0, 10.9 Hz), 3.72 (dd, 1H, J = 16.0, 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9, 153.2, 136.8, 134.0, 129.1, 128.2, 126.1, 126.2, 124.9, 122.7, 122.1, 120.7, 120.4, 120.0, 118.8, 81.2, 35.0.

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(4-methoxyphenyl)amide (**1b**)* Yield 19.5 %; m. p. 142.3–146.2 °C; IR 3061, 1681, 1245, 742 cm^{-1}; MS(ESI) m/z 342.2 (M + Na$^+$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 (d, 1H, J = 8.2 Hz), 7.86 (d, 1H, J = 8.2 Hz), 7.56–7.47 (m, 3H), 7.45 (d, 2H, J = 9.0 Hz), 7.36 (d, 1H, J = 8.2 Hz), 6.85 (d, 2H, J = 9.0 Hz), 5.47 (dd, 1H, J = 10.9, 6.5 Hz), 3.85 (dd, 1H, J = 16.0, 10.9 Hz), 3.78 (s, 2H), 3.78 (s, 3H) 3.71 (dd, 1H, J = 16.0, 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.6, 156.8, 153.2, 134.0, 129.9, 129.8, 128.2, 126.0, 122.7, 122.0, 121.8, 120.7, 120.4, 118.9, 114.2, 81.2, 55.5, 35.0.

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(4-chlorophenyl)amide (**1d**)* Yield 17.1 %; IR 3057, 1684, 743, 669 cm^{-1}; MS(ESI) m/z 346.1 (M + Na$^+$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.04 (d, 1H, J = 8.5 Hz), 7.87 (d, 1H, J = 8.5 Hz), 7.55 (t, 1H, J = 8.5 Hz), 7.52–7.49 (m, 4H), 7.36 (d, 1H, J = 8.5 Hz), 7.28 (d, 2H, J = 8.8 Hz), 5.47 (dd, 1H, J = 11.0, 6.2 Hz), 3.86 (dd, 1H, J = 16.0, 11.0 Hz), 3.70 (dd, 1H, J = 16.0, 6.2 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.0, 153.1, 135.3, 134.0, 129.9, 129.2, 129.1, 128.2, 126.1, 122.6, 122.2, 121.3, 120.6, 120.4, 118.7, 81.1, 35.0.

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(3,4-dichlorophenyl)amide (**1e**)* Yield 15.3 %; m. p. 146.9–150.8 °C; IR 2923, 1684, 743, 669 cm^{-1}; MS(ESI) m/z 358.2 (M + H$^+$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04 (d, 1H, J = 8.1 Hz), 7.87 (d, 1H, J = 8.0 Hz), 7.80 (s, 1H), 7.55–7.49 (m, 3H), 7.37–7.34 (m, 3H), 5.46 (dd, 1H, J = 11.0, 6.5 Hz), 3.86 (dd, 1H, J = 16.0, 11.0 Hz), 3.69 (dd, 1H, J = 16.0, 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.1, 153.0, 136.2, 134.0, 132.9, 130.5, 128.3, 128.1, 126.2, 122.6, 122.5, 122.3, 121.7 120.6, 120.4, 119.2, 118.6, 81.1, 35.0.

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(3,5-dichlorophenyl)amide (**1f**)* Yield 28.2 %; m. p. 169.9–170.8 °C; IR 2924, 1684, 750, 669 cm^{-1}; MS(ESI) m/z 358.1 (M + H$^+$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04 (d, 1H, J = 8.2 Hz), 7.87 (d, 1H, J = 8.2 Hz), 7.55 (td, 1H, J = 8.2, 1.7 Hz), 7.52–7.49 (m, 4H), 7.35 (d, 1H, J = 8.2 Hz), 7.10 (t, 1H, J = 1.7 Hz), 5.45 (dd, 1H, J = 11.0, 6.4 Hz), 3.85 (dd, 1H, J = 16.0, 11.0 Hz), 3.69 (dd, 1H, J = 16.0, 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.2, 153.0, 138.5, 135.3, 134.0, 128.3, 126.2, 126.1, 124.8, 122.5, 122.3, 120.6, 120.4, 118.6, 118.2, 81.0, 35.0.

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(3,5-bis(trifluoromethyl)phenyl)amide (**1g**)* Yield 9.8 %; m. p. 163.1–165.7 °C; IR 2924, 1683, 1177, 1131, 752 cm^{-1}; MS(ESI) m/z 448.2 (M + Na$^+$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.08–8.07 (m, 3H), 7.88 (d, 1H, J = 8.2 Hz), 7.63 (s, 1H), 7.58 (t, 1H, J = 8.2 Hz), 7.53–7.50 (m, 2H), 7.36 (d, 1H, J = 8.3 Hz), 5.50 (dd, 1H, J = 11.0, 6.7 Hz), 3.89 (dd, 1H, J = 16.0, 11.0 Hz), 3.72 (dd, 1H, J = 16.0, 6.7 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.6, 153.0, 138.2, 134.1, 132.7, 132.3, 128.3, 126.3, 126.2, 122.5, 122.4, 120.6, 120.4, 119.8 118.5 118.2, 81.0, 35.0.

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(2-trifluoromethylphenyl)amide (**1h**)* Yield 17.9 %; m. p. 154.8–158.7 °C; IR 3057, 1700, 1171, 1104, 744 cm^{-1}; MS(ESI) m/z 358.2 (M + H$^+$); ^{1}H NMR ($CDCl_3$,

400 MHz) δ 8.41 (d, 1H, $J = 8.3$ Hz), 8.05 (d, 1H, $J = 8.3$ Hz), 7.85 (d, 1H, $J = 8.3$ Hz), 7.59–7.53 (m, 3H), 7.49 (d, 1H, $J = 8.3$ Hz), 7.49 (t, 1H, $J = 8.3$ Hz), 7.35 (d, 1H, $J = 8.3$ Hz), 7.21 (t, 1H, $J = 8.3$ Hz), 5.50 (dd, 1H, $J = 11.2$, 5.6 Hz), 3.88 (dd, 1H, $J = 16.0$, 11.2 Hz), 3.72 (dd, 1H, $J = 16.0$, 5.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5, 153.0, 134.6, 134.5, 134.0, 133.0, 128.0, 126.2, 126.1, 124.5, 123.0, 122.5, 122.4, 122.2, 120.8, 120.5, 118.4, 81.2, 34.9.

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(4-trifluoromethylphenyl)amide (**1i**)* Yield 14.9 %; m. p. 135.0–137.8 °C; IR 3057, 1685, 1164, 1113, 741 cm^{-1}; MS(ESI) m/z 715.8 ($2\ M + H^+$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.06 (d, 1H, $J = 8.1$ Hz), 7.88 (d, 1H, $J = 8.1$ Hz), 7.69 (d, 2H, $J = 8.5$ Hz), 7.57 (d, 2H, $J = 8.5$ Hz), 7.56 (t, 1H, $J = 8.1$ Hz), 7.53–7.49 (m, 2H), 7.36 (d, 1H, $J = 8.1$ Hz), 5.49 (dd, 1H, $J = 11.0$, 6.4 Hz), 3.87 (dd, 1H, $J = 16.0$, 11.0 Hz), 3.71 (dd, 1H, $J = 16.0$, 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 153.0, 139.8, 134.0, 128.3, 126.5, 126.4, 126.3, 126.2, 126.1, 122.6, 122.3, 120.6, 120.4, 119.7, 118.7, 81.1, 35.0.

*General procedure for the synthesis of 2,3-dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(substitutedphenyl)amide analogs (**1c**, **1j** and **1k**)*

To a mixture of acid (1.0 eq), HOBt (1.0 eq) and EDCI (1.0 eq) in *N*,*N*-dimethylformamide was stirred for 10 min. The substituted aniline (1.0 eq) was added then after 10 min DIPEA (2.0 eq) at room temperature. After stirring for 12–16 h, the reaction mixture was washed with H_2O (3 × 10 mL) then acidified with 1 *N* HCl to pH 2. The mixture was extracted with EtOAc (3 × 10 mL) and the combined extracts were dried over anhydrous Na_2SO_4. After evaporation, the residue was purified by column chromatography (EtOAc/hexanes = 1:3–1:9).

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(3-chlorophenyl)amide (**1c**)* Yield 5.8 %; m. p. 108.3–113.9 °C; IR 2923, 1684, 745, 650 cm^{-1}; MS(ESI) m/z 346.2 ($M + Na^+$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 (d, 1H, $J = 8.2$ Hz), 7.86 (d, 1H, $J = 8.2$ Hz), 7.66 (t, 1H, $J = 2.0$ Hz), 7.58 (td, 1H, $J = 8.2$, 2.0 Hz), 7.50 (d, 1H, $J = 8.2$ Hz), 7.52 (td, 1H, $J = 8.2$, 2.0 Hz), 7.40 (dd, 1H, $J = 8.2$, 2.0 Hz), 7.35 (d, 1H, $J = 8.2$ Hz), 7.23 (t, 1H, $J = 8.2$ Hz), 7.10 (dd, 1H, $J = 8.2$, 2.0 Hz), 5.46 (dd, 1H, $J = 11.0$, 6.6 Hz), 3.82 (dd, 1H, $J = 16.0$, 10.8 Hz), 3.71 (dd, 1H, $J = 16.0$, 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.0, 153.1, 135.3, 134.7, 134.0, 129.9, 129.1, 128.2, 126.2, 126.1, 124.9, 122.6, 122.2, 121.3, 120.6, 120.4, 118.7, 118.0, 81.1, 35.0.

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(2-hydroxyphenyl)amide (**1j**)* Yield 4.7 %; m. p. 170.5–179.9 °C; IR 3361, 3056, 1648, 1120, 748 cm^{-1}; MS(ESI) m/z 328.3 ($M + Na^+$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.05 (d, 1H, $J = 7.9$ Hz), 7.87 (d, 1H, $J = 7.9$ Hz), 7.57–7.48 (m, 4H), 7.36 (d, 1H, $J = 8.2$ Hz), 7.12 (t, 1H, $J = 8.2$ Hz), 7.06 (d, 1H, $J = 8.2$ Hz), 7.00 (d, 1H, $J = 8.2$ Hz), 6.85 (t, 1H, $J = 8.2$ Hz), 5.54 (dd, 1H, $J = 11.0$, 6.4 Hz), 3.89 (dd, 1H, $J = 16.1$, 11.0 Hz), 3.72 (dd, 1H, $J = 16.1$, 6.4 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.5, 170.8, 153.0, 148.5, 134.1, 128.2, 127.5, 126.2, 126.1, 124.4, 122.5, 122.4, 122.3, 120.6, 120.4, 119.7, 118.5, 80.8, 35.1.

*2,3-Dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(4-hydroxyphenyl)amide (**1k**)* Yield 4.6 %; IR 3362, 2924, 1683, 1219, 735 cm^{-1}; MS(ESI) m/z 328.1 ($M + Na^+$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.04 (d, 1H, $J = 8.2$ Hz), 7.87 (d, 1H, $J = 8.2$ Hz), 7.55–7.47 (m, 3H), 7.39 (d, 2H, $J = 8.9$ Hz), 7.36 (d, 1H, $J = 8.2$ Hz), 6.78 (d, 2H, $J = 8.9$ Hz), 5.47 (dd, 1H, $J = 11.0$, 6.5 Hz), 3.85 (dd, 1H, $J = 16.1$, 11.0 Hz), 3.72 (dd, 1H, $J = 16.1$, 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.3, 169.9, 153.2, 134.0, 129.5, 128.2, 126.1, 125.4, 122.7, 122.3, 122.0, 120.7, 120.4, 118.8, 115.8, 81.2, 35.0.

*General procedure for the synthesis of 5-chloro-2,3-dihydronaphtho-[1,2-b]furan-2-carboxylic acid N-(substitutedphenyl)amide derivatives (**2** series)*

To a stirred solution of (2,3-dihydronaphtho[1,2-b]furan-2-yl)methanol (221 mg, 1.10 mmol) in THF/sat. $NaHCO_3$ (1/1, 2 mL) was added TEMPO (34.2 mg, 0.219 mmol), KBr (39.2 mg, 0.329 mmol), and NaOCl aq (4.8 mL) at 0 °C. After stirring for 1–6 h, the mixture was was washed with diethyl then the combined extracts were acidified with 1 *N* HCl to pH 2–3. The mixture was extracted with EtOAc (3 × 10 mL) and the combined extracts were dried over anhydrous Na_2SO_4. After evaporation, to a mixture of 5-chloro-2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxylic acid (1.0 eq) and 1,1′-carbodiimidazole (1.2 eq) in anhydrous THF was stirred for 1 h then substituted aniline (1.0 eq) was added at room temperature the reaction mixture was washed with H_2O (3 × 10 mL) then acidified with 1 *N*-HCl to pH 2. The mixture was extracted with EtOAc (3 × 10 mL) and the combined extracts were dried over anhydrous $MgSO_4$. After evaporation, the residue was purified by column chromatography (EtOAc/hexanes = 1:3–1:10).

*5-Chloro-2,3-dihydronaphtho[1,2-b]furan-2-carboxylic acid N-phenylamide (**2a**)* Yield 28.1 %; m. p. 173.3–176.7 °C; IR 3189, 3070, 1666, 755 cm^{-1}; MS(ESI) m/z 326.2

($M + Na^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.22 (m, 1H), 8.07 (m, 1H), 7.67 (s, 1H), 7.64–7.60 (m, 2H), 7.54 (d, 2H, $J = 8.7$ Hz), 7.32 (t, 2H, $J = 8.4$ Hz), 7.13 (t, 1H, $J = 8.4$ Hz), 5.49 (dd, 1H, $J = 10.8$, 6.6 Hz), 3.86 (dd, 1H, $J = 16.2$, 10.8 Hz), 3.73 (dd, 1H, $J = 16.2$, 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9, 153.2, 136.8, 134.0, 129.1, 128.2, 126.9, 126.1, 124.9, 122.7, 122.1, 120.7, 120.0, 119.7, 118.8, 81.2, 35.0.

*5-Chloro-2,3-dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(4-methoxyphenyl)amide (**2b**)* Yield 42.7 %; m. p. 156.3–157.1 °C; IR 3313, 1656, 1177, 736 cm^{-1}; MS(ESI) m/z 379.9 ($M + Na^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.20 (m, 1H), 7.84 (m, 1H), 7.62 (s, 1H), 7.59–7.53 (m, 2H), 6.89 (d, 2H, $J = 8.6$ Hz), 6.61 (d, 2H, $J = 8.6$ Hz), 5.31 (dd, 1H, $J = 11.1$, 5.9 Hz), 3.73 (dd, 1H, $J = 16.0$, 11.1 Hz), 3.71 (s, 1H), 3.56 (dd, 1H, $J = 16.0$, 5.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1, 158.3, 153.3, 131.7, 130.2, 129.6, 127.6, 127.4, 126.5, 125.0, 124.3, 121.3, 121.2, 119.7, 114.0, 81.3, 55.2, 34.6.

*5-Chloro-2,3-dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(3-chlorophenyl)amide (**2c**)* Yield 19.4 %; m. p. 177.5–179.8 °C; IR 3250, 1670, 1203, 737, 669 cm^{-1}; MS(ESI) m/z 390.2 ($M + Na^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.27 (m, 1H), 8.10 (m, 1H), 7.71–7.70 (m, 2H), 7.67–7.64 (m, 2H), 7.43 (d, 1H, $J = 8.2$ Hz), 7.29 (t, 1H, $J = 9.0$ Hz), 7.15 (d, 1H, $J = 8.2$ Hz), 5.52 (dd, 1H, $J = 10.9$, 6.5 Hz), 3.90 (dd, 1H, $J = 16.0$, 10.9 Hz), 3.75 (dd, 1H, $J = 16.0$, 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.5, 153.0, 137.8, 134.8, 131.9, 130.1, 127.8, 127.6, 127.0, 126.4, 125.1, 121.3, 121.1, 120.1, 119.6, 118.0, 114.8, 81.3, 34.7.

*5-Chloro-2,3-dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(4-chlorophenyl)amide (**2d**)* Yield 24.8 %; m. p. 147.8–154.3 °C; IR 3249, 1669, 1185, 757, 669 cm^{-1}; MS(ESI) m/z 380.0 ($M + Na^+$); ^1H NMR (MeOD, 400 MHz) δ 8.19–8.16 (m, 2H), 7.75 (s, 1H), 7.65 (d, 2H, J = 9.0 Hz), 7.63-7.59 (m, 2H), 7.34 (d, 2H, $J = 9.0$ Hz), 5.55 (dd, 1H, $J = 10.6$, 7.3 Hz), 3.81 (dd, 1H, $J = 16.1$, 10.6 Hz), 3.68 (dd, 1H, $J = 16.1$, 7.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.4, 153.1, 135.2, 131.9, 130.0, 129.1, 127.8, 127.6, 126.9, 126.4, 121.3, 121.1, 119.6, 119.0, 114.8, 81.3, 34.7.

*5-Chloro-2,3-dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(3,4-dichlorophenyl)amide (**2e**)* Yield 34.6 %; m. p. 184.9–188.3 °C; IR 3313, 1684, 1186, 738, 669 cm^{-1}; MS(ESI) m/z 416.4 ($M + Na^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.25 (m, 1H), 8.05 (m, 1H), 7.79 (s, 1H), 7.64–7.61 (m, 2H), 7.37–7.36 (d, 2H, $J = 1.4$ Hz), 5.48 (dd, 1H, $J = 10.9$, 6.5 Hz), 3.85 (dd, 1H, $J = 16.0$, 11.0 Hz), 3.70 (dd, 1H, $J = 16.0$, 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.5, 153.0, 136.1, 133.0, 130.6, 127.9, 127.6, 127.3, 127.0, 126.3, 125.3, 122.8, 121.7, 121.1, 121.0, 119.2, 144.9, 81.3, 34.8.

*5-Chloro-2,3-dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(3,5-dichlorophenyl)amide (**2f**)* Yield 21.7 %; m. p. 158.3–163.7 °C; IR 3306, 1684, 1187, 737, 668 cm^{-1}; MS(ESI) m/z 416.2 ($M + Na^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.23 (m, 1H), 8.04 (m, 1H), 7.67 (s, 1H), 7.64–7.61 (m, 2H), 7.52 (d, 2H, $J = 1.8$ Hz), 7.12 (t, 1H, $J = 1.8$ Hz), 5.47 (dd, 1H, $J = 11.0$, 6.5 Hz), 3.86 (dd, 1H, $J = 16.2$, 11.0 Hz), 3.70 (dd, 1H, $J = 16.2$, 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.6, 152.9, 138.4, 135.4, 131.9, 127.9, 127.6, 127.0, 126.3, 125.0, 121.3, 121.1, 119.4, 118.3, 115.0, 81.2, 34.7.

*5-Chloro-2,3-dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(3,5-ditrifluoromethylphenyl)amide (**2g**)* Yield 31.6 %; m. p. 156.1–158.4 °C; IR 3309, 1672, 1175, 1129, 760, 721 cm^{-1}; MS(ESI) m/z 482.92 ($M + Na^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.23 (m, 1H), 8.09–8.07 (m, 3H), 7.67 (s, 1H), 7.64–7.62 (m, 2H), 7.03 (s, 1H), 5.51 (dd, 1H, $J = 10.9$, 6.6 Hz), 3.88 (dd, 1H, $J = 16.2$, 10.9 Hz), 3.72 (dd, 1H, $J = 16.2$, 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.0, 153.3, 143.4, 138.1, 135.8, 132.7, 132.5, 132.0, 127.9, 127.7, 127.1, 126.3, 121.1, 119.8, 119.4, 115.1, 81.1, 34.7.

*5-Chloro-2,3-dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(2-trifluoromethylphenyl)amide (**2h**)* Yield 6.3 %; m. p. 153.2–163.9 °C; IR 3410, 1698, 1163, 1106, 759, 680 cm^{-1}; MS(ESI) m/z 414.2 ($M + H^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.40 (d, 1H, $J = 8.3$ Hz), 8.22 (m, 1H), 8.06 (m, 1H), 7.67 (s, 1H), 7.63–7.54 (m, 4H), 7.22 (t, 1H, $J = 7.7$ Hz), 5.51 (dd, 1H, $J = 10.9$, 5.8 Hz), 3.87 (dd, 1H, $J = 16.0$, 10.9 Hz), 3.75 (dd, 1H, $J = 16.0$, 5.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5, 153.0, 134.6, 134.0, 133.0, 128.0, 126.2, 126.1, 124.5, 123.0, 122.4, 122.3, 122.2, 120.4, 120.1, 119.9, 119.5, 118.4, 81.0, 34.9.

*5-Chloro-2,3-dihydronaphtho[1,2-b]furan-2-carboxylic acid N-(4-trifluoromethylphenyl)amide (**2i**)* Yield 27.6 %; m. p. 156.8–162.5 °C; IR 3275, 1698, 1122, 1118, 763, 683 cm^{-1}; MS(ESI) m/z 413.3 ($M + Na^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.25 (m, 1H), 8.07 (m, 1H), 7.68 (d, 1H, J = 8.2 Hz), 7.48 (s, 1H), 5.20 (dd, 1H, $J = 10.9$, 6.5 Hz), 3.87 (dd, 1H, $J = 16.0$, 10.9 Hz), 3.72 (dd, 1H, $J = 16.0$, 6.5 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 153.0, 139.8, 134.0, 128.3, 127.6, 126.4, 126.3, 126.2, 126.1, 122.6, 122.3, 120.6, 120.4, 119.7, 118.7, 81.4, 35.0.

Biology

Cell lines

The three human cancer cell lines, HCT-116, NCI-H23 and PC-3 were used in this study. All the cells were obtained from the National Cancer Institute, U.S.A. These cells were maintained in RPMI1640 media supplemented with 10 % fetal calf serum at 37 °C under a humidified atmosphere of 5 % CO_2.

SRB assay

The number of cells was measured indirectly using the sulforhodamine B (SRB) method according to the NCI (USA) protocol. Briefly, the cells were plated into a 96 well plate at a density of 2×10^3 cells per well. On the next day (day 0), the compounds of interest dissolved in DMSO/media were added in quadruplicate. The final concentration of each compound ranged from 1 to 10 μM and the final concentration of DMSO was <0.1 %. Seventy-two hours later, the cells were fixed with 10 % trichloroacetic acid (TCA) overnight at 4 °C. The TCA-treated cells were washed extensively with distilled water and dried in air. A SRB solution (0.4 % in 1 % acetic acid) was then added to the well at room temperature for 1 hour. The bound dye was dissolved in 10 mM Tris after washing the wells with 1 % acetic acid. The absorbances were measured at 690 nm using a micro plate reader. The absorbance of the day 0 sample was subtracted from the absorbance of the day 3 sample.

MTT assay

HCT-116 cancer cells were plated in 96-well plates (5×10^4 cells/well) per 100 mL medium, and subconfluent cells were subsequently treated with compounds (0–20 μg/mL). To determine the appropriate dose that is not cytotoxic to the cells, the cytotoxic effect was evaluated in the cells cultured for 72 h by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma–Aldrich, St. Louis, MO). Metabolic activity was quantified by measuring light absorbance at 540 nm.

Measurement of NF-κB transcriptional activity

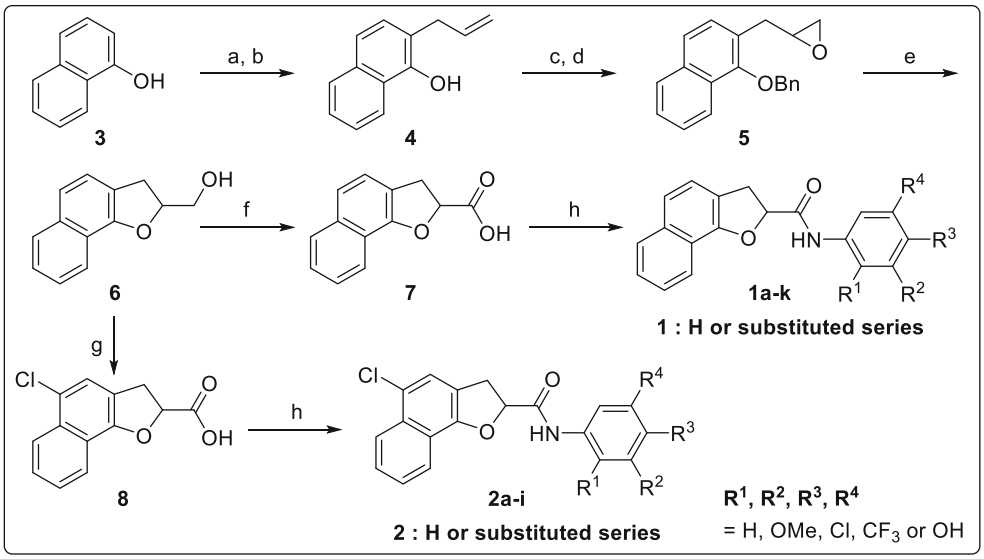
RAW 264.7 macrophages were stably transfected with NF-κB-SEAP-NPT plasmid and then treated with 1 μg/mL LPS plus sample for 16 h. Aliquots of the cell-free media were heated at 65 °C for 5 min, and then reacted with SEAP assay buffer (500 μM 4-methylumbelliferyl phosphate, 2 M diethanolamine, and 1 mM $MgCl_2$) in the dark at room temperature for 1 h. As a reporter, SEAP activity was measured as relative fluorescence units (RFU) with emission 449 nm and excitation 360 nm.

Results and discussion

Chemistry

Various substituents on the *N*-phenyl rings of the target compounds were introduced (H, OMe, Cl, CF_3, and OH); these substituents resulted in potent activity in the previous studies. In addition, we attempted to establish structure–activity relationships (SAR) according to the electronic and hydrophobic characteristics of the substituents. The positional effects of the substituents were also explored by examining biological activities of the compounds with substituents at various positions (2′-; 3′-; 4′-; 3′,4′-; and 3′,5′-) of the *N*-phenyl ring.

For the target naphtho-[1,2-*b*]furan-2-carboxylic acid *N*-(substitutedphenyl)amides (series **1** and **2**), the general synthetic procedure is outlined in Scheme 1. The commercially available starting material, 1-naphthol (**3**) was treated with 2-allylphenol in the presence of K_2CO_3 and then underwent Claisen rearrangement to obtain compound **4**. To prepare the dihydronaphthofuran ring, epoxidation-cyclization was performed with *m*-CPBA directly from compound **4**, but it resulted in low yield and side products. Therefore, we redesigned the synthetic strategy to include protection, epoxidation, and cyclization. The synthesis of compound **6** containing a dihydronaphthofuran ring was accomplished by benzylation using treatment of compound **4** with benzyl bromide and epoxidation with *m*-CPBA followed by cyclization with hydrogenolysis using 10 % Pd–C and H_2 gas. For 2,3-dihydronaphtho[1,2-*b*]furan-2-carboxylic acid (**7**), we attempted TEMPO oxidation with NaOCl. However, carboxylic acid **8** having a chloro group in the C-5 position was obtained instead of desired carboxylic acid **7** without a chloro group. Therefore, 2,3-dihydronaphtho[1,2-*b*]furan-2-carboxylic acid **7** was prepared using TEMPO and BAIB. After oxidation, 2,3-dihydronaphtho[1,2-*b*]furan-2-carboxylic acid *N*-(substitutedphenyl)amide derivatives (series **1**) were obtained by using the coupling agent CDI (1,1-carbonyldiimidazole) in anhydrous THF with aniline and its analogs with various substituents such as H, OH, OMe, Cl, and CF_3 groups. In addition, 5-chloro-2,3-dihydronaphtho[1,2-*b*]furan-2-carboxylic acid *N*-(substitutedphenyl)amide derivatives (series **2**) were synthesized from carboxylic acid **8** through treatment with 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and hydroxylbenzotriazole (HOBt) (Scheme 1).



Scheme 1 General methods to prepare carboxamide analogs. Reagents and conditions: *a*), allylbromide, K_2CO_3, acetone, reflux, 2 h, 98 %; *b*), xylene, reflux, 16 h, 88 %; *c*), BnBr, K_2CO_3, acetone, reflux, 8 h, 99 %; *d*), *m*-CPBA, CH_2Cl_2, reflux, 6 h, 73 %; *e*), 10 % Pd–C, H_2, K_2CO_3, Et_3N, MeOH, 8 h, 94 %; *f*), TEMPO, BAIB, CH_3CN–H_2O (50 % v/v), 6 h, 66 %; *g*), TEMPO, KBr, NaOCl, THF–sat. aq. $NaHCO_3$ (50 % v/v), 6 h; *h*), peptide coupling conditions: CDI, THF (**1 a–b, 1d–g, 1i,** and **2** series); HOBt, EDC, DIPEA, DMF (**1c, 1j,** and **1k**); EDC, THF (**1h**); 5–40 %

Biology

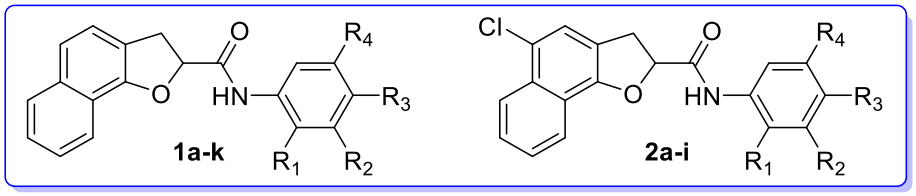
The newly synthesized library of 2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxamide analogs (**1a–k**) and 5-chloro-2,3-dihydronaphtho[1,2-*b*]furan-2-carboxamide analogs (**2a–i**) was evaluated for NF-κB inhibitory activity in LPS-stimulated RAW 264.7 cells (Table 1). Among the compounds of series **1**, only 2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxylic *N*-(4′-chlorophenyl) amide (**1d**) (IC_{50}: 26 μM) was more potent than the reference compounds **KL-1156** (IC_{50}: 37.2 μM) and **PDTC** (IC_{50}: 34.5 μM). In series **2** (chloro group in the C-5 position of the naphthofuran ring), 5-chloro-2,3-dihydronaphtho [1,2-*b*]furan-2-carboxylic *N*-phenylamide (**2a**) and 5-chloro-2,3-dihydronaphtho [1,2-*b*]furan-2-carboxylic *N*-(3′,5′-bis(trifluoromethyl)phenyl)amide (**2g**) had IC_{50} values of 27 μM and 12 μM, respectively. Compound **2g**, which contained 3′,5′-bistrifluoromethyl groups on the *N*-phenyl ring, exhibited the highest NF-κB inhibitory activity among the 2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxamide analogs; its inhibitory activity was approximately 3 times those of **KL-1156** and **PDTC**.

In the previous studies, chroman-2-carboxamide and (substituted)chroman-2-carboxamide analogs with a chloro group at C-4′ of the *N*-phenyl ring generally were most potent inhibitors of NF-κB in the respective series ($IC_{50} < 30$ μM) (Kwak et al. 2007, 2008, 2009b). Likewise, the best compound of series **1**, 2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxyamide (**1d**), had a chloro group at C-4′ of the *N*-phenyl ring (Fig. 2).

Unlike series **1** compounds, analogs having a chloro group at C-5 of the 2,3-dihydronaphtho[1,2-*b*]furan ring closely resembled the series of indoline-2-carboxamide, benzofuran-2-carboxamide, and 7-methychroman-2-carboxamde in NF-κB inhibitory activity (Kwak et al. 2009b, 2010; Choi et al. 2015). In compounds with a 5-membered ring system such as indoline or benzofuran, introduction of the trifluoromethyl group at C-3′ and C-5′ of the *N*-phenyl ring enhanced their NF-κB inhibitory activity. The data for the series of 5-chloro-2,3-dihydronaphtho[1,2-*b*]furan-2-carboxamide were consistent with the results of previous studies. Compound **2g**, which had a 5-membered ring and a trifluoromethyl group at C-3′ and C-5′ of the *N*-phenyl ring, exhibited most potent activity among series **2** compounds. In chroman analogs, only compounds containing a 3′,5′-bistrifluoromethyl group on the *N*-phenyl ring and a substitution of a methyl group at the C-7 position of the chroman moiety had NF-κB inhibitory activity (Fig. 2).

Among the compounds containing *N*-(4′-chlorophenyl)amide, the 2,3-dihydronaphtho[1,2-*b*]furan analog had as potent activity as the (substituted)chroman analog because of their structural similarity. However, the introduction of substituents (such as a chloro group) at the C-5 position of the naphthofuran moiety abolished NF-κB inhibitory activity. In the analogs having the 3′,5′-bistrifluoromethyl group, we confirmed that structural extension of the plane ring and introduction of a substituent at the C-5 positions of dihydrobenzofuran moieties preserved their NF-κB inhibitory activity. Interestingly, their activity was

Table 1 Inhibition of LPS-induced NF-κB activation by 2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxamide analogs



Substituent				No.	% inhibition at 30 μM (IC_{50}^{a})	No.	% inhibition at 30 μM (IC_{50}^{a})
R^1	R^2	R^3	R^4				
H	H	H	H	**1a**	46	**2a**	*56 (29 μM)*
H	H	OMe	H	**1b**	49	**2b**	50
H	Cl	H	H	**1c**	36	**2c**	37
H	H	Cl	H	**1d**	*57 (26 μM)*	**2d**	42
H	Cl	Cl	H	**1e**	19	**2e**	37
H	Cl	H	Cl	**1f**	43	**2f**	44
H	CF_3	H	CF_3	**1g**	30	**2g**	*88 (12 μM)*
CF_3	H	H	H	**1h**	41	**2h**	31
H	H	CF_3	H	**1i**	39	**2i**	36
OH	H	H	H	**1j**	38		
H	H	OH	H	**1k**	42		
KL-1156 (Ref)						IC_{50}: 37.2 μM	
PDTC (Ref)						IC_{50}: 34.5 μM	

Compounds having more potent activity than others are in italics

[a] IC_{50} values are means of three experiments of the concentrations exhibiting 50 % inhibition of LPS-induced NF-κB transcriptional activity

improved when a substituent was added at the C-7 position of the chroman moiety and the C-5 position of the dihydronaphthofuran moiety.

Recent study reported that naphthofuran compounds regulate HNF4α, which is associated with proliferation, progression, and metastasis of hepatocellular carcinoma (Le Guevel et al. 2009). In particular, compound **2g** inhibits liver tumor growth through inhibition of STAT3 (Kwon et al. 2015). Therefore, we also evaluated synthesized compounds (**1** and **2** series) for in vitro cytotoxicity against human cancer cell lines HCT-116, NCI-H23, and PC-3 by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and sulforhodamine B assays.

In the MTT assay, six 2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxamide analogs (**1a, 1d–g,** and **1i**) and eight 5-chloro-2,3-dihydronaphtho[1,2-*b*]furan-2-carboxamide analogs (**2a** and **2c–i**) inhibited cell growth of the colon cancer cell line HCT-116 by more than 50 % at 25 μg/ml (Table 2). 2,3-dihydronaphtho [1,2-*b*]furan-2-carboxylic *N*-(3′,4′-dichlorophenyl)amide (**1e**) and 5-chloro-2,3-dihydronaphtho[1,2-*b*]furan-2-carboxylic *N*-(3′,5′-bis(trifluoromethyl)phenyl) amide (**2g**) exhibited the best activity among the compounds of the respective series (9.70 and 16.11 % cell viability, respectively). In addition, compounds **1e** and **2g** exhibited concentration-dependent inhibition of cancer cell growth (Fig. 3). Compound **1e** showed a GI_{50} value of 7.97 μg/mL and compound **2g** had a GI_{50} value of 2.68 μg/mL against HCT-116 cells (Table 2). Most importantly, the inhibitory activity of compound **2g** was more potent than those of 5-fluorouracil (5-FU) and oxaliplatin, which were used as reference anticancer compounds.

However, cell growth inhibitory activity of the tested compounds was not directly related to their NF-κB inhibitory activity. Interestingly, only 5-chloro-2,3-dihydronaphtho [1,2-*b*]furan-2-carboxylic *N*-(3′,5′-bis(trifluoromethyl) phenyl)amide (**2g**), which was reported as an outstanding inhibitor of STAT3 (Kwon et al. 2015), had not only potent NF-κB inhibitory activity but also cytotoxicity against HCT-116 cells.

We also evaluated the growth inhibitory effects of series **1** and **2** against human cancer cell lines NCI-H23 (lung) and PC-3 (prostate) by using the sulforhodamine B assay (Table 3). Three 2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxamide analogs (**1c**, **1e**, and **1h**) and two 5-chloro-2,3-dihydronaphtho[1,2-*b*]furan-2-carboxamide analogs (**2f** and **2g**) exhibited considerable activities against NCI-H23 cells. Compounds **1c** (3′-Cl on the *N*-phenyl ring), **1e** (3′,4′-diCl), and **1h** (2′-CF_3) had moderate cytotoxicity with GI_{50} values of 4.50 μM, 3.15 μM, and 5.65 μM, respectively. Compounds **2f** (3′,5′-diCl) and **2 g** (3′,5′-diCF_3) showed

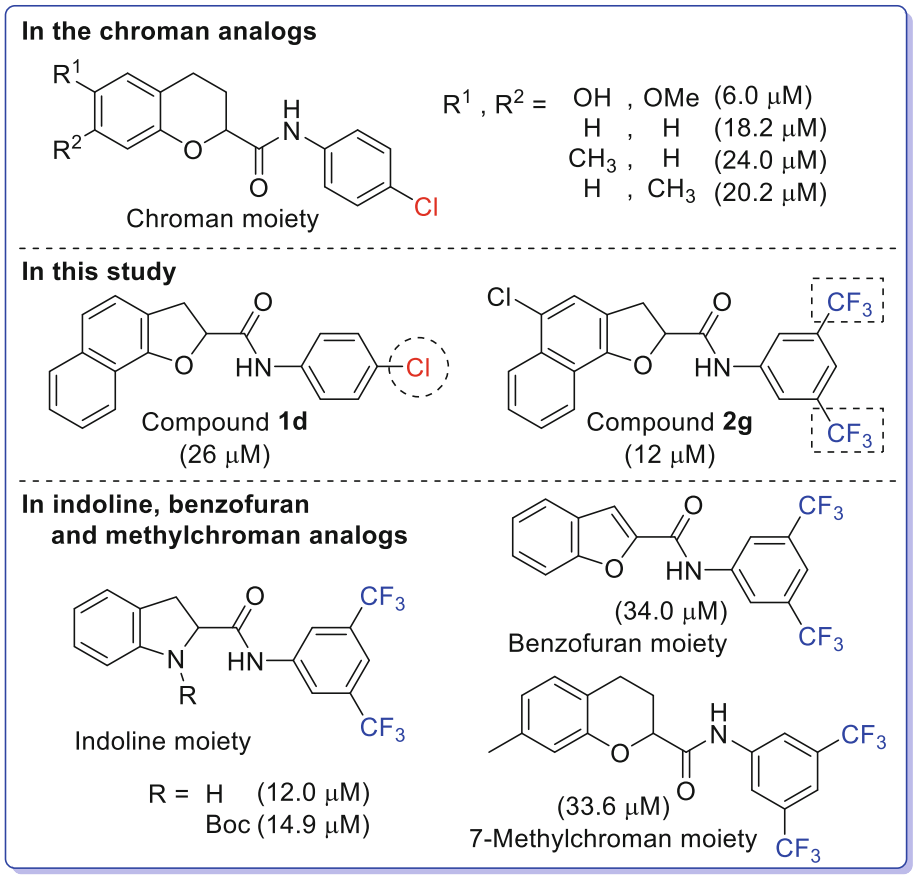


Fig. 2 NF-κB inhibitory activity (IC_{50} values) of compounds possessing a 4′-chloro or 3′,5′-bistrifluoromethyl group on the *N*-phenyl ring. (Kwak et al. 2007, 2008, 2009a, b, 2010; Choi et al. 2015)

Table 2 Viability of HCT-116 colorectal carcinoma cells treated with 2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxylic acid *N*-(substitutedphenyl)amide analogs

No.	Viability at 25 μg/mL (%) (GI_{50} value)[a]	No.	Viability at 25 μg/mL (%) (GI_{50} value)[a]
1a	*36.25*	**2a**	*47.11*
1b	78.12	**2b**	57.51
1c	58.27	**2c**	*23.05*
1d	*33.26*	**2d**	*32.93*
1e	*9.70 (7.97 μg/mL)*	**2e**	*44.37*
1f	*46.63*	**2f**	*40.66*
1g	*44.92*	**2g**	*16.11 (2.68 μg/mL)*
1h	54.57	**2h**	*43.84*
1i	*22.38*	**2i**	*44.68*
1j	74.64	**5-FU**	36.59 (at 10 μg/mL)
1k	82.18	**Oxaliplatin**	55.94 (at 10 μg/mL)

Compounds having more potent activity than others are in italics

[a] GI_{50} values are means of three experiments and correspond to the agent's concentration causing a 50 % decrease in net cell growth

cytotoxic activity with GI_{50} values of 3.80 μM and 3.08 μM, respectively. Compound **2 g** had the best activity, but its activity against NCI-H23 cells was lower than those of pyrrolidine, Bay-11-7082, and Adriamycin, which were used as reference compounds. When tested with PC-3 cells, only three compounds inhibited cancer cell growth: compound **1e** (GI_{50} = 6.51 μM), **2f** (5.57 μM), and **2g** (3.30 μM). Overall, compound **2g** had the best cytotoxic activity among the prepared compounds, although it was not as good as those of the reference compounds (Table 3).

To explore the SAR in order to achieve potent cytotoxic activities against the three cancer cell lines, we substituted various groups (R^1, R^2, R^3, and R^4) on the planar *N*-phenyl ring of the carboxamide functionality. Compounds **1b**, **1j**,

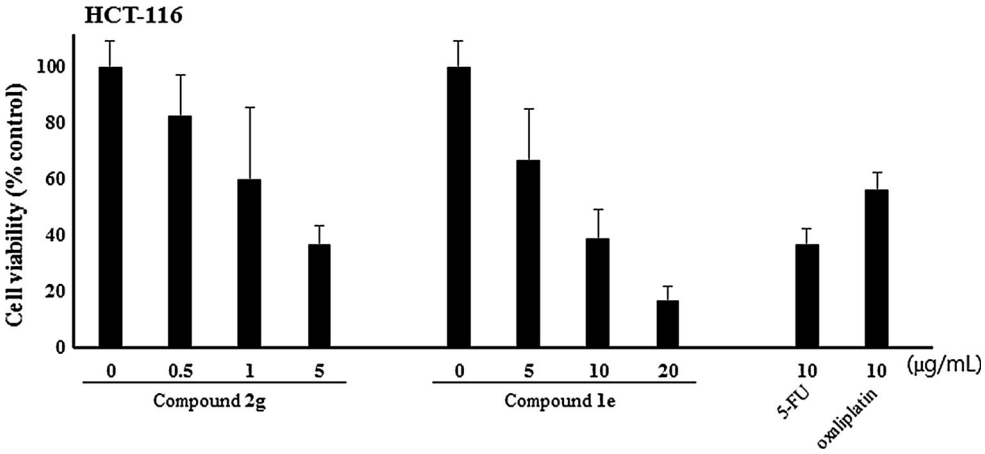


Fig. 3 Effect of compounds **2g** and **1e** on viability of colon cancer cells (HCT-116). 5-FU and oxaliplatine were used as reference compounds. After treatment for 72 h, the cells were harvested by trypsinization and stained with MTT. The data are expressed as the mean ± SD of three experiments

Table 3 Anticancer activities of 2,3-dihydronaphtho-[1,2-*b*]furan analogs against human cancer cell lines

No.	NCI-H23 (GI_{50}, μM)[a]	PC-3 (GI_{50}, μM)[a]	No.	NCI-H23 (GI_{50}, μM)[a]	PC-3 (GI_{50}, μM)[a]
1a	>10	>10	**2a**	>10	>10
1b	>10	>10	**2b**	>10	>10
1c	*4.50*	>10	**2c**	>10	>10
1d	>10	>10	**2d**	>10	>10
1e	*3.15*	*6.51*	**2e**	>10	>10
1f	>10	>10	**2f**	*3.80*	*5.57*
1g	>10	>10	**2g**	*3.08*	*3.30*
1h	5.65	>10	**2h**	>10	>10
1i	>10	>10	**2i**	>10	>10
1j	>10	>10			
1k	>10	>10			
	Pyrrolidine			0.079	0.205
	Bay-11-7082			1.078	1.028
	ADR			0.086	0.037

Compounds having more potent activity than others are in italics

[a] GI_{50} values are means of three experiments and correspond to the agent's concentration causing a 50 % decrease in net cell growth

1k, and **2b** with introduced electron-donating groups such as methoxy or hydroxyl groups exhibited lower cytotoxic activity than compounds with other substituents. Compounds with anticancer activity generally had two introduced electron-withdrawing groups (such as chloro or trifluoro groups) on the *N*-phenyl ring. For instance, compounds **1e** with 3′,4′-dichloro group, **2f** with 3′,5′-dichloro group, and **2g** with 3′,5′-bisfluoromethyl group on the *N*-phenyl ring were more potent than compounds with other substituents. In the derivatives of compounds **2f** and **2 g** that were cytotoxic against NCI-H23 and PC-3 cell lines, the substituents were located at the C-3′ and C-5′ positions of the *N*-phenyl ring. Only compounds **1e** and **2g** exhibited potent cell growth inhibitory activity against all three lines tested (HCT-116, NCI-H23, and PC-3), and compound **2g** had the best cytotoxic activity. Moreover, compound **2g** inhibited the transcription factors NF-κB and STAT3 (Kwon et al. 2015).

In conclusion, a series of 2,3-dihydronaphtho-[1,2-*b*]furan-2-carboxamide analogs (**1a–k**) and 5-chloro-2,3-dihydronaphtho[1,2-*b*]furan-2-carboxamide analogs (**2a–i**) was designed and synthesized from commercially available starting materials via the well-known Claisen rearrangement, epoxidation-cyclization, oxidation, and amidation reactions using CDI or EDC. Compounds **1d** and **2g** exhibited the best NF-κB inhibitory activity in LPS-stimulated RAW 264.7 cells among the compounds of the respective series. Compound **1d**, which had a 4′-chloro group on the *N*-phenyl ring, exhibited NF-κB inhibitory activity because of its structural similarity to a previously reported (substituted)chroman analog. In contrast, as SAR of indoline, benzofuran and 7-methylchroman moiety, compound **2g** with the introduced 5-chloro group on the naphthofuran ring and 3′,5′-bisfluoromethyl groups on the *N*-phenyl ring had the best NF-κB inhibitory activity. The in vitro anticancer activities of these analogs were evaluated

against three human cancer cell lines: HCT-116 (colon), NCI-H23 (lung), and PC-3 (prostate). 5-Chloro-2,3-dihydronaphtho[1,2-*b*]furan-2-carboxylic *N*-(3′,5′-bis(trifluoromethyl)phenyl)amide (**2g**) exhibited the most potent anticancer activity against the three tested cell lines. 2,3-dihydronaphtho [1,2-*b*]furan-2-carboxylic *N*-(3′,4′-dichlorophenyl)amide (**1e**) also exhibited promising growth inhibitory activities against all these cell lines. In the SAR studies, two electron-withdrawing groups at the planar *N*-phenyl rings of compounds **1e**, **2f**, and **2g** resulted in more potent anticancer activities than those of all other analogs. However, cytotoxicity of these analogs was not directly related to their NF-κB inhibitory activities. Only compound **2g** exhibited both outstanding anticancer and NF-κB inhibitory activities. Our SAR studies have encouraged us to investigate additional novel lead scaffolds exhibiting potent anticancer activity through inactivation of NF-κB; this work is in progress.

Acknowledgments This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2013R1A1A2009381), Medical Research Center Program (2008-0,062,275) and the research grant of Chungbuk National University in 2014.

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