
# Design, Synthesis, and Biological Evaluation of Benzofuran- and 2,3-Dihydrobenzofuran-2-carboxylic acid *N*-(substituted)phenylamide Derivatives as Anticancer Agents and Inhibitors of NF-κB

Minho Choi, Hyeju Jo, Hyun-Jung Park, Arepalli Sateesh Kumar, Joonkwang Lee, Jieun Yun, Youngsoo Kim, Sang-bae Han, Jae-Kyung Jung, Jungsook Cho, Kiho Lee, Jae-Hwan Kwak, Heesoon Lee

PII: S0960-894X(15)00382-0
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.04.050
Reference: BMCL 22633

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 30 October 2014
Revised Date: 6 April 2015
Accepted Date: 17 April 2015

Please cite this article as: Choi, M., Jo, H., Park, H-J., Kumar, A.S., Lee, J., Yun, J., Kim, Y., Han, S-b., Jung, J-K., Cho, J., Lee, K., Kwak, J-H., Lee, H., Design, Synthesis, and Biological Evaluation of Benzofuran- and 2,3-Dihydrobenzofuran-2-carboxylic acid *N*-(substituted)phenylamide Derivatives as Anticancer Agents and Inhibitors of NF-κB, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.04.050

Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

# Design, Synthesis, and Biological Evaluation of Benzofuran- and 2,3-Dihydrobenzofuran-2-carboxylic acid *N*-(substituted)phenylamide Derivatives as Anticancer Agents and Inhibitors of NF-κB

Minho Choi [a], Hyeju Jo [a], Hyun-Jung Park [a], Arepalli Sateesh Kumar [a], Joonkwang Lee [a], Jieun Yun[b], Youngsoo Kim [a], Sang-bae Han [a], Jae-Kyung Jung [a], Jungsook Cho [c], Kiho Lee [d], Jae-Hwan Kwak [e] *, Heesoon Lee [a] *

[a]*Department of Pharmacy, Chungbuk National University, Chungbuk 362-763, Republic of Korea.*
[b]*Korea Research Institute of Bioscience and Biotechnology, Ochang 363-883, Republic of Korea.*
[c]*College of Pharmacy, Dongguk University, Goyang 410-773, Republic of Korea.*
[d]*College of Pharmacy, Korea University, Sejong 339-700, Republic of Korea.*
[e]*College of Pharmacy, Kyungsung University, Busan 608-736, Republic of Korea.*

ARTICLE INFO

*Article history:*
Received
Revised
Accepted
Available online

*Keywords:*
Anticancer activity
Inhibition of NF-κB transcriptional activity
Benzofuran and 2,3-dihydrobenzofuran scaffolds.

ABSTRACT

With the aim of developing novel scaffolds as anticancer agents and inhibitors of NF-κB activity, 60 novel benzofuran- and 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(substituted)phenylamide derivatives (**1a–s, 2a–k, 3a–s,** and **4a–k**) were designed and synthesized from the reference lead compound **KL-1156,** which is an inhibitor of NF-κB translocation to the nucleus in LPS-stimulated RAW 264.7 macrophage cells. The novel benzofuran- and 2,3-dihydrobenzofuran-2-carboxamide derivatives exhibited potent cytotoxic activities (measured by the sulforhodamine B assay) at low micromolar concentrations against six human cancer cell lines: ACHN (renal), HCT15 (colon), MM231 (breast), NUGC-3 (gastric), NCI-H23 (lung), and PC-3 (prostate). In addition, these compounds also inhibited LPS-induced NF-κB transcriptional activity. The +M effect and hydrophobic groups on the *N*-phenyl ring potentiated the anticancer activity and NF-κB inhibitory activity, respectively. However, according to the results of structure–activity relationship studies, only benzofuran-2-carboxylic acid *N*-(4′-hydroxy)phenylamide (**3m**) was the lead scaffold with both an outstanding anticancer activity and NF-κB inhibitory activity. This novel lead scaffold may be helpful for investigation of new anticancer agents that act through inactivation of NF-κB.



Cancer is a large family of diseases resulting from uncontrolled cell growth.[1] Gene mutations, one of the causes of abnormal cell growth, are induced by DNA damage as a result of exposure to carcinogens, such as chemicals, viruses, ultraviolet light, and radicals.[2-5] Another cause of carcinogenesis is aberrant activation of cell signaling pathways involving hormones, cytokines, and chemokines.[6-8] Among them, nuclear factor-κB (NF-κB), an inducible transcription factor, is present in an inactive form in the cytoplasm in most cell types.[9-11] Upon activation by external stimuli or abnormal signaling, NF-κB translocates to the nucleus and binds DNA. NF-κB binding to DNA regulates the expression of genes related to inflammation and cell growth.[12,13] In various types of human cancer, dysregulated NF-κB specifically modulates the transcription of genes controlling cell survival, expression of cell adhesion molecules, cell differentiation, and cell growth. Therefore, it has been suggested that the inhibitors of NF-κB function might also be useful for developing antitumor agents.[14-16]

As part of our continuous efforts to design and develop novel scaffolds for anticancer activity and NF-κB inhibitory activity,[17-21] we recently reported a series of novel indoline-2-carboxylic acid *N*-(substituted)phenylamide derivatives[21] and explored their NF-κB inhibitory activities and cytotoxicity against various human cancer cell lines.

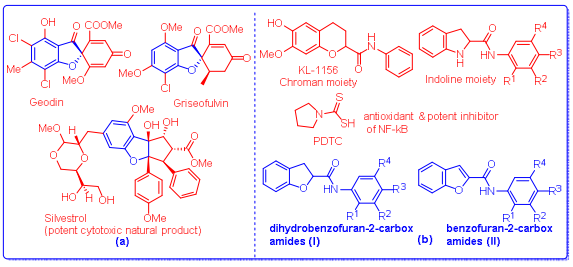
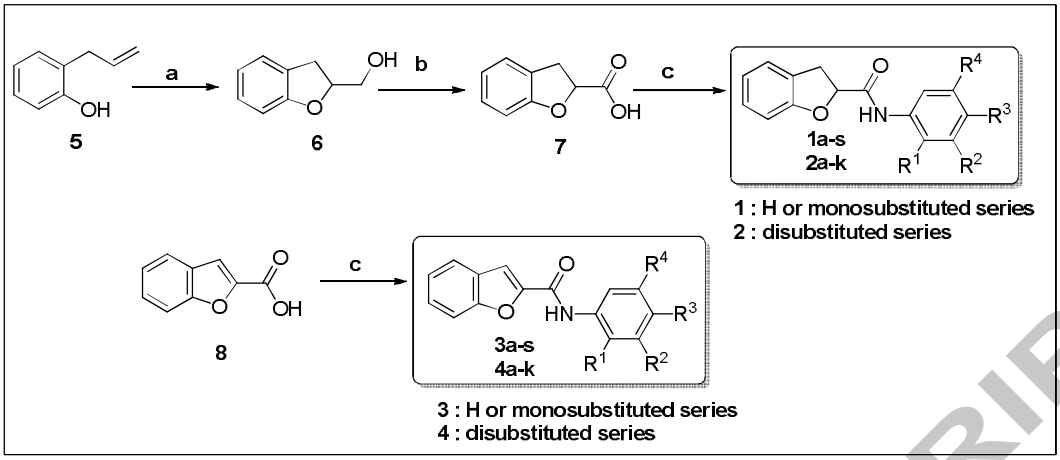



**Figure 1.** (a) Biologically active scaffolds of benzofuran and dihydrobenzofuran (b) Structures of chroman, indoline, 2,3-dihydrobenzofuran, and benzofuran moieties..

* Corresponding authors. Tel.: +82-43-261-2811; fax: +82-43-268-2732; e-mail: medchem@chungbuk.ac.kr (H. Lee)
+82-51-663-4889; e-mail: jhkwak@ks.ac.kr (J. H. Kwak)




**Scheme 1.** General methods to prepare carboxamide derivatives. Reagents and conditions: (a) *m*-CPBA, DCM, 6 h then $K_2CO_3$, MeOH, 12 h, 87%; (b) TEMPO, KBr, aq. NaOCl, THF, saturated aq. $NaHCO_3$, 3 h, 69%; (c) CDI, Ar-$NH_2$, THF, 12 h, 5–95%.

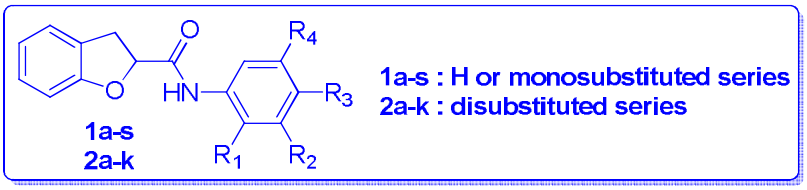
These derivatives were designed on the basis of the parent lead compound KL-1156, an inhibitor of NF-κB translocation to the nucleus in LPS-stimulated RAW 264.7 macrophage cells;[17] the synthesized compounds exhibited outstanding inhibitory effects on NF-κB activation as well as anticancer activities in comparison with KL-1154. Those indoline derivatives that had NF-κB inhibitory activity generally also exhibited cytotoxicity, although it was not directly proportional to their NF-κB inhibitory activity.[21]

The core structures of benzofuran and dihydrobenzofuran are a class of most important heterocyclic motifs prevailing in natural products with pharmacological potential. For instance, griseofulvin and its analogs have been found to exhibit antiviral and anticancer activities.[22-24] The cyclopenta[*b*]benzofuran silvestrol, shown in **Figure 1(a)**, is a very potent cytotoxic natural product against several human cancer cell lines.[25-27] Therefore, in this study we have designed and synthesized novel scaffolds of benzofuran- and 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(substituted) phenyl amide derivatives (**I** and **II** as shown in **Figure 1(b)**) to identify the compounds exhibiting most potent anticancer and NF-κB inhibitory activities. To establish the structure–activity relationships (SAR), various substituents (H, $NO_2$, $CH_3$, $OCH_3$, OH, Cl, and $CF_3$) were introduced into the *N*-phenyl rings of the target compounds according to their electronic and hydrophobic characteristics. 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-*; *3,5-*; and *2,5-*) on the phenyl ring.

To obtain 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(substituted)phenyl amides (**1a–s** and **2a–k**), we used commercially available 2-allylphenol, which undergoes epoxidative cyclization upon treatment with *m*-CPBA; this reaction provided **6** in 87% yield. Among various oxidation conditions for the conversion of alcohol **6** to carboxylic acid **7**, the condition using TEMPO and NaOCl in tetrahydrofuran afforded 2,3-dihydrobenzofuran-2-carboxylic acid **7** in 69% yield within 3 h.[28] After oxidation, 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(substituted)phenyl amide derivatives (**1a–s** and **2a–k**) were obtained by using the coupling reagent 1,1′-carbonyldiimidazole (CDI) [29] in anhydrous tetrahydrofuran with aniline and its derivatives (which have various substituents such as H, $NO_2$, $CH_3$, $OCH_3$, OH, Cl, and $CF_3$ groups; **Scheme 1**). In a similar fashion, benzofuran-2-carboxylic acid *N*-(substituted)phenyl amide derivatives (**3a–s** and **4a–k**) were synthesized from commercially available benzofuran-2-carboxylic acid (**8**) (**Scheme 1**). The newly synthesized library of benzofuran and 2,3-dihydrobenzofuran carboxamides (**1a–s**, **2a–k**, **3a–s**, and **4a–k**) was evaluated for *in vitro* cytotoxicity against human cancer cell lines ACHN (renal), HCT15 (colon), MM231 (breast), NUGC-3 (gastric), NCI-H23 (lung), and PC-3 (prostate) by using sulforhodamine B (SRB) assay[30] (**Tables 1** and **2**); **KL-1156**, pyrrolidine dithiocarbamate (**PDTC**)[17], and doxorubicin (**ADR**) were used as reference compounds. Only some of the derivatives exhibited considerable biological activities in these cell lines. Among 2,3-dihydrobenzofuran derivatives (**1a–s** and **2a–k**), compound **1p** bearing 4′-Cl on the *N*-phenyl ring showed moderate cytotoxicity only against HCT15 cell line. As shown in **Table 1**, cytotoxicties of some 2,3-dihydrobenzofuran-2-carboxamdie derivatives against PC-3 cell line were more potent than those against other tested cell lines. In particular, 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(4′-nitrophenyl)amide (**1d**) and 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(3′-hydroxyphenyl)amide (**1l**) exhibited very promising cytotoxicity against PC-3 cell line with $GI_{50}$ values of 1.86 and 3.97 μM, respectively. Compounds **1s**, **2a**, and **2f** also had moderate cytotoxicity against PC-3 cells (60%, 55%, and 38% cell viability at 10 μM, respectively). Compound **1l** was also mildly cytotoxic against MM231 (63% cell viability at 10 μM) and NCI-H23 cell lines (60% cell viability at 10 μM).

In contrast to series **1** and **2**, benzofuran-2-carboxylic acid *N*-(substituted)phenyl amide derivatives (**3a–s** and **4a–k**) generally showed low cytotoxicity, with the exception of benzofuran-2-carboxylic acid *N*-(4′-hydroxyphenyl)amide (**3m**), benzofuran-2-carboxylic acid *N*-(2′-hydroxyphenyl)amide (**3k**), and benzofuran-2-carboxylic acid *N*-(2′-chlorophenyl)amide (**3o**) (**Table 2**). Compound **3m** exhibited outstanding growth inhibitory activity against all tested cancer cell lines with $GI_{50}$ values of 2.74 μM (ACHN), 2.37 μM (HCT15), 2.20 μM (MM231), 2.48 μM (NUGC-3), 5.86 μM (NCI-H23), and 2.68 μM (PC-3). At 10 μM concentration, compound **3k** showed 58% HCT15 cell viability and compound **3o** also exhibited moderate anticancer activities against HCT15 and NCI-H23 cell lines (52% and 61% cell viability, respectively).

**Table 1.** Anticancer activities of 2,3-dihydrobenzofuran derivatives against six human cancer cell lines.

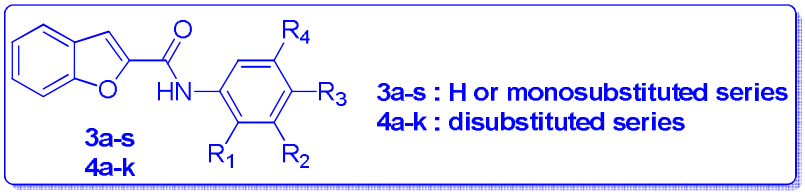



| Substituents | | | | No. | % cell viability at 10 μM | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $R^1$ | $R^2$ | $R^3$ | $R^4$ | | ACHN | HCT15 | MM231 | NCI-H23 | NUGC-3 | PC-3 [a] ($GI_{50}$) |
| H | H | H | H | **1a** | 95±4 | 88±4 | 101±6 | 93±4 | 87±3 | 92±5 |
| $NO_2$ | H | H | H | **1b** | 108±4 | 94±5 | 92±2 | 97±4 | 89±5 | 89±3 |
| H | $NO_2$ | H | H | **1c** | 109±9 | 83±2 | 92±8 | 95±3 | 83±4 | 77±8 |
| H | H | $NO_2$ | H | **1d** | 93±7 | 81±6 | 85±2 | 77±4 | 86±5 | **10±5 (1.86 μM)** |
| $CH_3$ | H | H | H | **1e** | 108±3 | 88±8 | 103±7 | 91±4 | 91±1 | 87±5 |
| H | $CH_3$ | H | H | **1f** | 145±8 | 99±1 | 95±6 | 98±1 | 108±2 | 96±9 |
| H | H | $CH_3$ | H | **1g** | 134±9 | 100±1 | 96±5 | 105±1 | 112±3 | 80±4 |
| $OCH_3$ | H | H | H | **1h** | 134±5 | 100±2 | 89±5 | 111±1 | 111±5 | 88±9 |
| H | $OCH_3$ | H | H | **1i** | 135±6 | 99±2 | 80±8 | 102±2 | 106±2 | 89±8 |
| H | H | $OCH_3$ | H | **1j** | 140±5 | 89±3 | 94±4 | 102±2 | 108±6 | 81±3 |
| OH | H | H | H | **1k** | 134±7 | 102±1 | 83±5 | 102±9 | 103±3 | 109±6 |
| H | OH | H | H | **1l** | 125±6 | 97±3 | **63±3** | **60±8** | 110±7 | **4±4 (3.97 μM)** |
| H | H | OH | H | **1m** | 122±4 | 88±6 | 91±3 | 106±3 | 102±4 | 98±7 |
| Cl | H | H | H | **1n** | 87±5 | 102±1 | 81±7 | 104±4 | 104±6 | 96±8 |
| H | Cl | H | H | **1o** | 134±6 | 97±2 | 80±3 | 101±3 | 101±4 | 91±6 |
| H | H | Cl | H | **1p** | 143±4 | **55±4** | 100±6 | 89±2 | 100±2 | 71±6 |
| $CF_3$ | H | H | H | **1q** | 136±7 | 98±2 | 98±6 | 102±1 | 103±7 | 74±8 |
| H | $CF_3$ | H | H | **1r** | 134±3 | 95±4 | 102±3 | 100±1 | 93±9 | 69±8 |
| H | H | $CF_3$ | H | **1s** | 124±3 | 96±5 | 82±7 | 97±1 | 99±6 | **60±5** |
| $CH_3$ | H | H | $CH_3$ | **2a** | 129±6 | 88±3 | 88±5 | 103±1 | 81±4 | **55±3** |
| H | $CH_3$ | H | $CH_3$ | **2b** | 136±3 | 91±8 | 97±6 | 97±5 | 95±6 | 94±7 |
| H | $CH_3$ | $CH_3$ | H | **2c** | 137±6 | 97±2 | 86±9 | 92±1 | 99±5 | 94±6 |
| $OCH_3$ | H | H | $OCH_3$ | **2d** | 126±5 | 97±4 | 89±5 | 99±5 | 95±7 | 81±7 |
| H | $OCH_3$ | H | $OCH_3$ | **2e** | 123±3 | 91±4 | 78±7 | 95±5 | 96±2 | 73±4 |
| H | $OCH_3$ | $OCH_3$ | H | **2f** | 127±1 | 92±3 | 71±4 | 101±5 | 87±3 | **38±4** |
| Cl | H | H | Cl | **2g** | 101±4 | 77±4 | 74±3 | 90±3 | 105±5 | 84±3 |
| H | Cl | H | Cl | **2h** | 89±6 | 98±6 | 89±7 | 99±2 | 114±2 | 89±6 |
| H | Cl | Cl | H | **2i** | 109±3 | 88±6 | 98±7 | 94±3 | 107±3 | 99±9 |
| $CF_3$ | H | H | $CF_3$ | **2j** | 105±4 | 81±6 | 98±6 | 87±9 | 99±4 | 88±5 |
| H | $CF_3$ | H | $CF_3$ | **2k** | 94±9 | 92±5 | 105±6 | 92±3 | 105±5 | 96±7 |
| **KL-1156 (Ref.)** | | | | | 109±3 | 105±7 | 81±5 | 86±3 | 99±7 | 90±10 |
| **ADR** | | **[a]$GI_{50}$** | | | 0.20 | 0.45 | 0.56 | 0.40 | 0.19 | 0.91 |
| **PDTC** | | **[a]$GI_{50}$** | | | 0.23 | 0.13 | 0.16 | 0.10 | 0.14 | 0.17 |

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

To explore the SAR towards potent cytotoxic activities of anticancer agents against the six 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. We observed that derivatives containing positive mesomeric effect (+M effect) groups such as hydroxyl (-OH), methoxy (-$OCH_3$), and chloro (-Cl) groups exhibited very promising anticancer activities on tested cancer cell lines (**Tables 1** and **2**). For instance, compounds **1l**, **1p**, **2f**, **3k**, **3m**, and **3o** were more potent than compounds with other substituents.

**Table 2**. Anticancer activities of benzofuran derivatives against six human cancer cell lines.

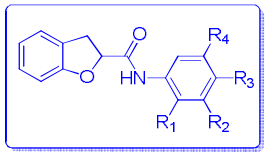



| Substituents | | | | No. | % cell viability at 10 μM | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $R^1$ | $R^2$ | $R^3$ | $R^4$ | | **ACHN** [a]($GI_{50}$) | **HCT15** [a]($GI_{50}$) | **MM231** [a]($GI_{50}$) | **NCI-H23** [a]($GI_{50}$) | **NUGC-3** [a]($GI_{50}$) | **PC-3** [a]($GI_{50}$) |
| H | H | H | H | **3a** | 89±8 | 75±1 | 94±7 | 98±2 | 97±6 | 83±6 |
| $NO_2$ | H | H | H | **3b** | 91±4 | 73±4 | 102±5 | 95±5 | 95±4 | 92±3 |
| H | $NO_2$ | H | H | **3c** | 93±7 | 88±4 | 111±8 | 99±3 | 98±5 | 95±7 |
| H | H | $NO_2$ | H | **3d** | 94±5 | 84±4 | 111±8 | 101±4 | 95±4 | 87±9 |
| $CH_3$ | H | H | H | **3e** | 106±6 | 93±5 | 101±6 | 89±2 | 98±6 | 100±5 |
| H | $CH_3$ | H | H | **3f** | 93±5 | 87±1 | 106±5 | 101±1 | 99±3 | 94±3 |
| H | H | $CH_3$ | H | **3g** | 96±3 | 82±3 | 105±7 | 104±1 | 95±3 | 103±10 |
| $OCH_3$ | H | H | H | **3h** | 95±5 | 94±4 | 97±9 | 98±2 | 99±2 | 106±4 |
| H | $OCH_3$ | H | H | **3i** | 105±5 | 87±3 | 127±9 | 105±1 | 99±4 | 106±8 |
| H | H | $OCH_3$ | H | **3j** | 109±3 | 87±5 | 110±9 | 101±3 | 102±2 | 111±8 |
| OH | H | H | H | **3k** | 84±2 | **58±3** | 85±4 | 86±5 | 84±4 | 68±3 |
| H | OH | H | H | **3l** | 84±6 | 85±3 | 100±9 | 95±1 | 87±3 | 90±10 |
| H | H | OH | H | **3m** | **10±3 (2.74 μM)** | **18±3 (2.37 μM)** | **-32±1 (2.20 μM)** | **25±2 (2.48 μM)** | **47±3 (5.86 μM)** | **-31±1 (2.68 μM)** |
| Cl | H | H | H | **3n** | 76±7 | 75±4 | 76±10 | 91±2 | 86±1 | 91±4 |
| H | Cl | H | H | **3o** | 69±6 | **52±5** | 80±9 | **61±9** | 83±2 | 95±1 |
| H | H | Cl | H | **3p** | 89±3 | 68±4 | 96±5 | 92±1 | 91±4 | 84±3 |
| $CF_3$ | H | H | H | **3q** | 98±2 | 99±10 | 114±9 | 100±2 | 93±3 | 95±5 |
| H | $CF_3$ | H | H | **3r** | 83±10 | 75±3 | 105±4 | 92±2 | 79±5 | 96±3 |
| H | H | $CF_3$ | H | **3s** | 79±2 | 80±8 | 89±9 | 89±2 | 75±4 | 74±7 |
| $CH_3$ | H | H | $CH_3$ | **4a** | 82±8 | 94±4 | 102±7 | 94±1 | 88±3 | 98±3 |
| H | $CH_3$ | H | $CH_3$ | **4b** | 101±3 | 79±3 | 94±6 | 99±3 | 93±5 | 94±4 |
| H | $CH_3$ | $CH_3$ | H | **4c** | 103±5 | 95±6 | 92±5 | 100±3 | 91±5 | 92±4 |
| $OCH_3$ | H | H | $OCH_3$ | **4d** | 102±3 | 97±3 | 101±5 | 103±5 | 91±3 | 106±10 |
| H | $OCH_3$ | H | $OCH_3$ | **4e** | 102±2 | 95±8 | 106±5 | 102±4 | 90±3 | 92±5 |
| H | $OCH_3$ | $OCH_3$ | H | **4f** | 99±3 | 92±5 | 81±6 | 102±4 | 90±3 | 98±2 |
| Cl | H | H | Cl | **4g** | 96±3 | 81±5 | 101±9 | 96±4 | 89±2 | 97±5 |
| H | Cl | H | Cl | **4h** | 99±4 | 95±5 | 102±3 | 100±3 | 93±4 | 99±8 |
| H | Cl | Cl | H | **4i** | 95±4 | 96±5 | 80±6 | 100±1 | 102±5 | 113±9 |
| $CF_3$ | H | H | $CF_3$ | **4j** | 84±6 | 88±1 | 98±10 | 77±5 | 89±4 | 95±6 |
| H | $CF_3$ | H | $CF_3$ | **4k** | 95±5 | 98±3 | 98±2 | 101±6 | 98±3 | 110±9 |
| **KL-1156 (Ref.)** | | | | | 109±3 | 105±7 | 81±5 | 86±3 | 99±7 | 90±10 |
| **ADR (Ref)** [a]$GI_{50}$ | | | | | 0.2 | 0.45 | 0.56 | 0.40 | 0.19 | 0.91 |
| **PDTC** [a]$GI_{50}$ | | | | | 0.23 | 0.13 | 0.16 | 0.10 | 0.14 | 0.17 |

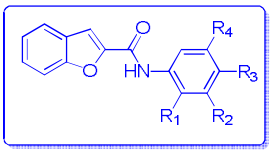
[a] $GI_{50}$ values are the means of three experiments and correspond to the agent's concentration causing a 50% decrease in net cell growth.

Among all 60 derivatives, benzofuran-2-carboxylic acid *N*-(4′-hydroxyphenyl)amide (**3m**) exhibited the most potent anticancer activities against all tested human cancer cell lines. The growth inhibitory activities were not directly related to the number and position of substituents on the *N*-phenyl ring.

**Table 3.** Inhibition of LPS-induced NF-κB activation by 2,3-dihydrobenzofuran and benzofuran carboxamide derivatives.




1a-s : H or monosubstituted series
2a-k : disubstituted series




3a-s : H or monosubstituted series
4a-k : disubstituted series

| Substituents | | | | 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** | 10.0 | **3a** | 44.0 |
| $NO_2$ | H | H | H | **1b** | 28.5 | **3b** | 25.5 |
| H | $NO_2$ | H | H | **1c** | 40.0 | **3c** | 11.0 |
| H | H | $NO_2$ | H | **1d** | 34.0 | **3d** | 34.5 |
| $CH_3$ | H | H | H | **1e** | 38.0 | **3e** | 46.0 |
| H | $CH_3$ | H | H | **1f** | 25.0 | **3f** | 38.0 |
| H | H | $CH_3$ | H | **1g** | 34.5 | **3g** | 34.5 |
| $OCH_3$ | H | H | H | **1h** | 29.0 | **3h** | 41.0 |
| H | $OCH_3$ | H | H | **1i** | 16.5 | **3i** | 34.5 |
| H | H | $OCH_3$ | H | **1j** | 22.5 | **3j** | 39.5 |
| OH | H | H | H | **1k** | 20.5 | **3k** | 23.0 |
| H | OH | H | H | **1l** | 17.0 | **3l** | 41.0 |
| H | H | OH | H | **1m** | 18.5 | **3m** | **62.0** |
| Cl | H | H | H | **1n** | 37.5 | **3n** | 49.5 |
| H | Cl | H | H | **1o** | 43.5 | **3o** | 37.0 |
| H | H | Cl | H | **1p** | 41.5 | **3p** | 43.0 |
| $CF_3$ | H | H | H | **1q** | 44.5 | **3q** | **57.5** |
| H | $CF_3$ | H | H | **1r** | 30.5 | **3r** | 47.5 |
| H | H | $CF_3$ | H | **1s** | 41.0 | **3s** | 34.5 |
| $CH_3$ | H | H | $CH_3$ | **2a** | 22.0 | **4a** | **58.0** |
| H | $CH_3$ | H | $CH_3$ | **2b** | 34.0 | **4b** | **70.0** |
| H | $CH_3$ | $CH_3$ | H | **2c** | 44.5 | **4c** | **70.0** |
| $OCH_3$ | H | H | $OCH_3$ | **2d** | 39.5 | **4d** | 23.0 |
| H | $OCH_3$ | H | $OCH_3$ | **2e** | 24.0 | **4e** | **63.0** |
| H | $OCH_3$ | $OCH_3$ | H | **2f** | 21.5 | **4f** | 40.0 |
| Cl | H | H | Cl | **2g** | 42.5 | **4g** | 36.5 |
| H | Cl | H | Cl | **2h** | **75.0** | **4h** | 20.0 |
| H | Cl | Cl | H | **2i** | **65.0 (23.0 μM)** | **4i** | 40.0 |
| $CF_3$ | H | H | $CF_3$ | **2j** | 34.0 | **4j** | 28.5 |
| H | $CF_3$ | H | $CF_3$ | **2k** | 25.0 | **4k** | **51.5 (34.0 μM)** |
| | | | | **KL-1156 (Ref.)** | | $IC_{50}$ : 37.2 μM | |
| | | | | **PDTC (Ref)** | | $IC_{50}$ : 34.5 μM | |

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

We were delighted to observe that compounds **1a–s**, **2a–k**, **3a–s**, and **4a–k** exhibited more potent anticancer activities than the parent lead compound **KL-1156** and reported indoline-2-carboxylic acid *N*-(substituted)phenyl amide derivatives.[21] We also evaluated the 60 newly synthesized derivatives of 2,3-dihydrobenzofuran-2-carboxamide and benzofuran-2-carboxamide for their inhibitory effects on NF-κB in LPS-stimulated RAW 264.7 macrophage cells; **KL-1156** and **PDTC** were used as reference compounds (**Table 3**). In series **1** and **2**, 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(3′,5′-dichloro phenyl)amide (**2h**) and 2,3-dihydrobenzofuran-2-carboxylic *N*-(3′,4′-dichlorophenyl)amide (**2i**) showed very potent inhibitory activities ($IC_{50}$ values under 30 μM). In series **3** and **4**, compounds **3m**, **3q**, **4a**, **4b**, **4c**, **4f**, and **4k** exhibited in particular potent inhibitory activities ($IC_{50}$ values under 30 μM) (**Table 3**). Among all derivatives, 2,3-dihydrobenzofuran-

2-carboxylic acid *N*-(3,5-dichlorophenyl)amide (**2h**) exhibited the most potent NF-κB inhibitory activity (75%). 2,3-dihydrobenzofuran-2-carboxylic *N*-(3′,4′-dichlorophenyl)amide (**2i**) (65% NF-κB inhibition) was 1.5 times more potent ($IC_{50}$: 23.0 μM) than reference compounds KL-1156 ($IC_{50}$: 37.2 μM) and PDTC ($IC_{50}$: 34.5 μM). Taken together, the data of the current study identify several derivatives of **1a–s**, **2a–k**, **3a–s**, **4a–k** with more potent NF-κB inhibitory activities in LPS-stimulated RAW 264.7 cells than those of the parent lead compound **KL-1156** and PDTC.

In relation to the SAR towards NF-κB inhibitory activity, compounds possessing hydrophobic groups on the *N*-phenyl ring were most potent as NF-κB inhibitors. For instance, di-chloride, dimethyl, dimethoxy, and trifluoromethyl groups (**2i**, **2h**, **3q**, **4a**, **4b**, **4c**, **4e**, and **4k**) exhibited most potent NF-κB inhibitory activities (**Table 3**). On the other hand, among the compounds having hydrophilic 4′-OH group on the *N*-phenyl ring, only **3m** exhibited promising NF-κB inhibitory activity. Collectively, the data from the current study indicate that only benzofuran-2-carboxylic acid *N*-(4′-hydroxyphenyl)amide (**3m**) can be considered as a novel derivative for inhibiting the transcription factor NF-κB, which also has potent anticancer activity against all six human cancer cell lines. Interestingly, several of the synthesized novel benzofuran- and 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(substituted)phenyl amide derivatives exhibited more potent anticancer activities and inhibition of NF-κB transcriptional activity than the parent reference compound **KL-1156**.

In conclusion, a series of novel benzofuran and 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(substituted)phenyl amide derivatives (**1a–s**, **2a–k, 3a–s**, and **4a–k)** was designed and synthesized from commercially available starting materials *via* well-known epoxidative cyclization and amidation reactions using CDI. We evaluated the *in vitro* anticancer activities of these derivatives against six human cancer cell lines. Benzofuran-2-carboxylic acid *N*-(4′-hydroxyphenyl)amide (**3m**) exhibited the most potent anticancer activity against all tested cell lines. 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(3′-hydroxyphenyl)-amide (**1l**) exhibited promising growth inhibitory activities against MM231, NCI-H23, and PC-3 cell lines. 2,3-dihydrobenzofuran-2-carboxylic acid *N*-(4′-nitrophenyl)-amide (**1d**) showed potent growth inhibitory activity against PC-3 cell line. 2,3-dihydrobenzofuran derivatives (**1a–s** and **2a–k**) had higher cytotoxicity against PC-3 than against other tested cancer cell lines. SAR studies revealed that substitutions at the planar *N*-phenyl ring of compounds **1l**, **1p**, **2f**, **3k**, **3m**, and **3o** (which have +M effect groups) exhibited more potent anticancer activities than all other derivatives. However, cytotoxicity of these derivatives was not directly related to their NF-κB inhibitory activities. Only benzofuran-2-carboxylic acid *N*-(4′-hydroxy)phenylamide (**3m**) exhibited both outstanding anticancer and NF-κB inhibitory activities. Our SAR studies have encouraged us to investigate more novel lead scaffolds exhibiting most potent anticancer activity through inactivation of NF-κB; this work is in progress.

## Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (NRF-2013R1A1A2009381), and by Medical Research Center Program (2008-0062275).

## Supplementary data

Supplementary data (Methods, materials, and general preparation procedures and analytical data) associated with this article can be found in the online version at http://dx.doi.org/

## References and notes

1. Khan, Z.; Bisen, P. S. *Biochem. Biophys. Res. Acta* **2013**, *1836*, 123–145
2. Nowell, P.C. *Cancer Research* **1986**, *46*, 2203–2207
3. Vogelstein, B.; Kinzler, K.W. Nature Medicine **2004**, *10*, 789–799.
4. Ichihashi, M.; Ueda, M..; Budiyanto, A.; Bito, T.; Oka, M.; Fukunaga, K.; Tsuru, T.; Horikawa, T. *Toxicology* **2003**, *189*, 21–39
5. Clemens, M.R. *Klinische Wochenschrift* **1991**, *69*, 1123–1134.
6. Mantovani, A.; Allavena, P.; Sica, A.; Balkwill, F. *Nature* **2008**, *454*, 436–444
7. Clayton, P.E.; Banerjee, I.; Murray, P.G.; Renehan, A.G. *Nature Reviews Endocrinology* **2011**, *7*, 11–24
8. Porta, C.; Riboldi, E.; Sica, A. *Cancer Lett.* **2011**, *305*, 250–262.
9. Hayden, M.S.; Ghosh, S. *Genes & Development* **2004**, *18*, 2195–2224.
10. Bonizzi G.; Karin M. *Trends Immunol.* **2004**, *25*, 280-288.
11. Shinkura, R.;Kitada, K.; Matsuda, F.; Tashiro, K.; Ikuta, K.; Suzuki, M.; Kogishi, K.; Serikawa, T.; Honjo, T. *Nat. Genet.* **1999**, *22*, 74–77.
12. Perkins, N.D.; Gilmore, T.D. *Cell Death and Differentiation* **2006**, *13*, 759–772.
13. Greten F. R.; Eckmann L.; Greten T. F.; Park J. M.; Li Z. W.; Egan L. J.; Kagnoff M. F.; Karin M. *Cell* **2004**, *118*, 285–296.
14. Perkins N. D. *Trends Cell. Biol.* **2004**, *14*, 64–69.
15. Luo J. L.; Kamata H.; Karin M. *J. Clin. Invest.* **2005**, *115*, 2625-2632.
16. Ryan K. M.; Ernst M. K.; Rice N. R.; Vousden K. H. *Nature* **2000**, *404*, 892–897
17. Kim, B. H.; Reddy, A. M.; Lee, K. H.; Chung, E. Y.; Cho, S. M.; Lee, H.; Min, K. R.; Kim, Y. *Biochem. Biophys. Res. Commun.* **2004**, *325*, 223–228.
18. Kwak, J.H.; Kim, B.H.; Jung, J.K.; Kim, Y.; Cho, J.; Lee, H. *Arch. Pharm. Res.* **2007**, *30*, 210–1215
19. Kwak, J. H.; Won, S. W.; Kim, T. J.; Roh, E.; Kang, H. Y.; Lee, H. W.; Jung, J. K.; Hwang, B. Y.; Kim, Y.; Cho, J.; Lee, H. *Arch. Pharm. Res.* **2008**, *31*, 133–141.
20. Kwak, J. H.; Won, S. W.; Kim, T. J.; Yi, W.; Choi, E. H.; Kim, S. C.; Park, H.; Roh, E.; Jung, J. K.; Hwang, B. Y.; Hong, J. T.; Kim, Y.; Cho, J.; Lee, H. *Arch. Pharm. Res.* **2009**, *32*, 167–175.
21. Kwak, J. H.; Kim, Y.; Park, H.; Jang, J. Y.; Lee, K.K.; Yi, W.; Kwak, J. A.; Park, S.G.; Kim, H.; Lee, K.; Kang, J. S.; Han, S.B.; Hwang, B.Y.; Hong, J.T.; Jung, J.K.; Kim, Y.; Cho, J.; Lee, H. *Bioorg. Med. Chem. Lett.* **2010,** *20*, 4620–4623.
22. Singh P.; Rathinasamy K.; Mohan R.; Panda D. *IUBMB Life* **2008**, *60*, 368-375.
23. Use of Griseofulvin for Inhibiting the Growth of Cancers. The Proctor & Gamble Company. *Patent WO9705870A2*, (*1997*).
24. Ho, Y. S.; Duh, J. S.; Jeng, J. H.; Wang, Y. J.; Liang, Y.; Lin, C. H.; Tseng, C. J.; Yu, C. F.; Chen, R. J.; Lin, J. K. *Int. J. Cancer* **2001**, *91*, 393.
25. Ohse T.; Ohba S.; Yamamoto T.; Koyano T.; Umezawa K.; J. Nat. Prod. 1996, 59, 650–652.
26. Cencic, R.; Carrier, M.; Galicia-Vázquez, G.; Bordeleau, M. E.; Sukarieh, R.; Bourdeau, A.; Brem, B.; Teodoro, J. G.; Greger, H.; Tremblay, M. L.; Porco, J. A., Jr.; Pelletier, J. *PLoS One* **2009**, *4*, e5223
27. Kim S.; Hwang B. Y.; Su B. N.; Chai H.; Mi Q.; Kinghorn A. D.; Wild R.; Swanson S. M. *Anticancer Res.* **2007**, *27*, 2175-2183.
28. Ramadas, S.; Krupadanam, G. L. D. *Tetrahedron: Asymmetry* **2000**, *11*, 3375–3393.
29. Montalbetti, C.A.G.N.; Falque, V. *Tetrahedron* **2005**, *61*, 10827–10852.
30. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.;. Warren, J.T.; Bokesch, H.; Kenny, S.; Boyd, M.R. *Journal of the National Cancer Institute* **1990**, *82*, 1107–1112.

31. *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 cell-free media were heated at 65°C for 5 min and then reacted with SEAP (secreted alkaline phosphatase) assay buffer (500 μM 4-methylumbelliferyl phosphate, 2 M diethanolamine, and 1 mM $MgCl_2$) in the dark at room temperature for 1 h. SEAP activity was measured as a reporter as relative fluorescence units (emission at 449 nm, excitation at 360 nm).
32. *In vitro cytotoxicity assay* was performed using the number of cells measured indirectly by the sulforhodamine B (SRB) method according to the NCI (USA) protocol (see ref. 13a). Briefly, cells were plated into a 96 well plate at a density of $2 \times 10^3$ 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 nM 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, washed extensively with distilled water and dried in air. SRB solution (0.4% in 1% acetic acid) was then added to each well at room temperature for 1 h. The wells were washed with 1% acetic acid and bound dye was dissolved in 10 mM Tris. The absorbance was 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.
33. *Benzofuran-2-carboxylic acid N-(4-hydroxyphenyl)amide (3m):* Yield 13%; m. p. 200–208 °C; FTIR 3347, 1655, 1529, 833 $cm^{-1}$; $^1H$ NMR (500 MHz, $CD_3OD$) $\delta$ 7.75 (d, 1H, $J$ = 7.8 Hz), 7.64 (d, 1H, $J$ = 7.8 Hz), 7.58 (s, 1H), 7.51 (d, 2H, $J$ = 8.9 Hz), 7.48 (t, 1H, $J$ = 7.8 Hz), 7.34 (t, 1H, $J$ = 7.8 Hz), 6.80 (d, 2H, $J$ = 8.9 Hz, Ar-H); $^{13}C$ NMR (100 MHz, $CDCl_3$) $\delta$ 161.72, 159.22, 154.76, 134.44, 131.05, 126.46, 126.31, 126.04, 125.76, 124.81, 123.10, 112.67, 112.01; HRMS (ESI) m/z calc'd for $C_{15}H_{10}NO_3$ $[M-H]^-$ : 252.0666, found 252.0667.

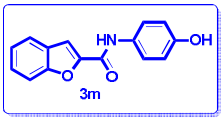
## Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Leave this area blank for abstract info.

# Design, Synthesis and Biological Evaluation of Benzofuran- and 2,3-Dihydrobenzofuran-2-carboxylic acid *N*-(substituted) phenylamide Derivatives as Anticancer Agents and inhibitors of NF-κB

Minho Choi [a], Hyeju Jo [a], Hyun-Jung Park [a], Arepalli Sateesh Kumar [a], Joonkwang Lee [a], Jieun Yun [b], Youngsoo Kim [a], Sang-bae Han [a], Jae-Kyung Jung [a], Jungsook Cho [c], Kiho Lee [d], Jae-Hwan Kwak [e*] Heesoon Lee [a*]




1. Exhibited potent anticancer activities on 6 human cancer cell lines
2. Exhibited potent Inhibition of NF-kB transcriptional activity