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# Design and Synthesis of 3,4-Dihydro-2*H*-benzo[*h*]chromene Derivatives as Potential NF-κB Inhibitors

Minho Choi [a], Young-Sik Hwang [a], Arepalli Sateesh Kumar [a], Hyeju Jo [a], Yeongeun Jeong [a], Yunju Oh [a], Joonkwang Lee [a], Jieun Yun [b], Youngsoo Kim [a], Sang-bae Han [a], Jae-Kyung Jung [a], Jungsook Cho [c] and Heesoon Lee [a] *

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ABSTRACT

A novel class of NF-κB inhibitors were designed and synthesized based on KL-1156 (6-Hydroxy-7-methoxychroman-2-carboxylic acid phenyl amide) which is unambiguously considered to be a promising inhibitor for the translocation step of NF-κB. Especially in this study we focused on the modifying the chroman moiety of KL-1156 into four parts for exploring the SAR studies linked with physical properties of substituents resulted the development of novel **1a-k**, **2a-f**, **3a-d** and **4a-d** derivatives of 3,4-dihydro-2*H*-benzo[*h*]chromene. From the SAR studies we were very delightfully identified that several new *N*-aryl-3,4-dihydro-2*H*-benzo[*h*]chromene-2-carboxamide derivatives (**1a-k**) exhibited good inhibitory activity and anti-proliferative activity than parent lead compound KL-1156, among them **1i** exhibited outstanding inhibitory effect on LPS-induced NF-κB transcriptional activity and anti-proliferative activity on NCI-H23 lung cancer cell lines than KL-1156.



In 1986 Baltimore *et al.,* discovered NF-κB as a factor in the nucleus of B cells that binds to the enhancer of the kappa light chain of immunoglobulin and it also has been characterized as a lymphoid specific protein.[1] Since then an intensive research have been dedicated to the study of NF-κB signaling, approximately more than 25,000 publications have been reported so far. NF-κB is also found in almost all animal cell types and is involved in cellular responses to stimuli such as stress cytokines, oxidized LDL, ultraviolet irradiation, free radical, and viral or bacterial antigens.[2-6] NF-κB is constitutively active in most tumor cell lines, whether derived from solid tumors or hematopoietic tumors. It is infrequently established to be constitutively active in normal cells except for proliferating T cells, thymocytes, B cells, monocytes, and astrocytes.[7] Constitutively active NF-κB has been identified not only in human cell lines but also in tumor tissues derived from patients with multiple myeloma, chronic myelogenous leukemia, acute lymphocyte leukemia, and acute myelogenous leukemia, breast and prostate cancers.[8-11]

Consequently, it has been suggested and very useful that inhibitors of NF-κB function both as anti-inflammatory agents and as antitumor agents.[12] Over the years, having interest on developing the novel inhibitors of NF-κB, our group reported a multiplicity of chroman derivatives having inhibitory activity of NF-κB[13-16] based on compound KL-1156 (6-hydroxy-7-methoxychroman-2-carboxylic acid phenyl amide) (Figure 1), an inhibitor of translocation to the nucleus in LPS-stimulated macrophage RAW 264.7 cells.[13] In the investigation studies of inhibitors of NF-κB, we had been recognized that the parent lead compound KL-1156 having phenolic -OH group and short alkyl groups of the chroman moiety have no significant effect for enhancement of inhibitory activity of NF-κB. Therefore we synthesized a series of indoline-2-carboxylic acid *N*-(substituted) phenylamide derivatives and they were also evaluated for cytotoxicity against various cancer cell lines.[17]

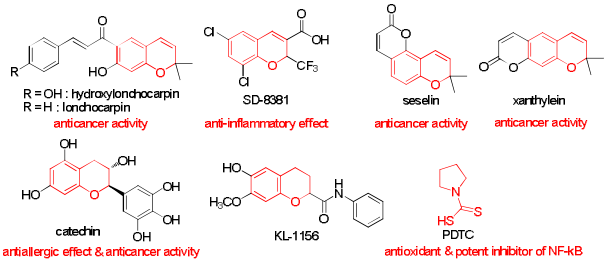


**Figure 1.** Representative examples of chroman (Benzopyran) scaffolds containing biologically active molecules.

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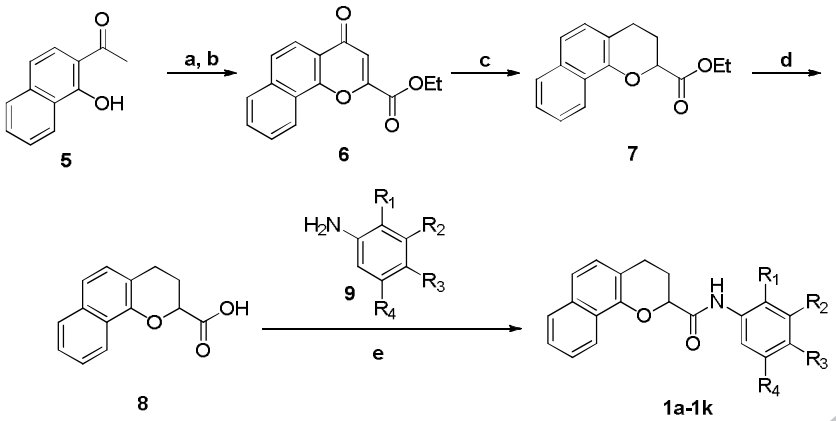
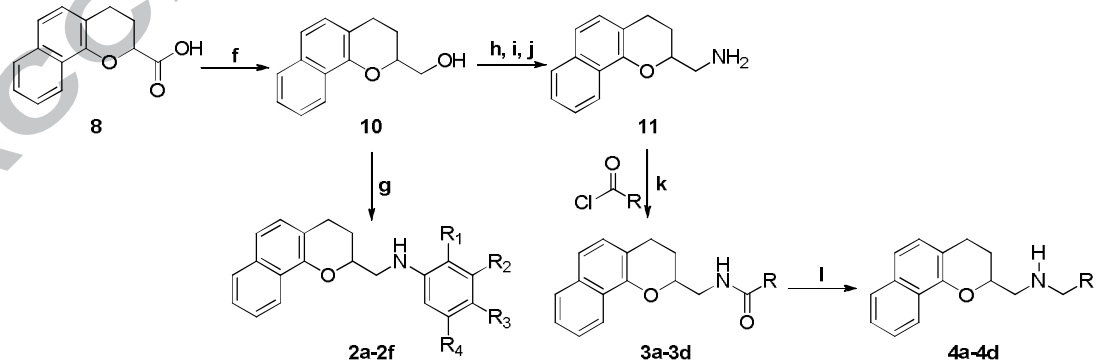



**Scheme 1.** General methods to prepare 1 series. Reagents and conditions: (a) diethyloxalate, NaOEt, EtOH, reflux, 3 h (b) EtOH, HCl (c) $H_2$, 10% Pd/C, EtOH, 24 h (d) KOH, isopropylalcohol/$H_2O$, reflux, 3h (after 4 steps yield 50%) (e) CDI, THF, 12 h, 52-75%

Furthermore, chroman (benzopyran) scaffold also frequently found in various biologically active molecules as shown in **Figure 1**. Hence these scaffolds are also traditionally valuable and to establish a systematic SAR of pyranyl moiety in chroman scaffolds, expansion of aromatic core of chroman to benzo[*h*]chromene could be considered. Furthermore we have introduced amide, carboxamide, aryl and alkyl amine functionalities to the core structure of benzo[*h*]chromene which could provide high-water solubility and exert beneficial pharmacokinetic effects. Thus, as part of our ongoing efforts to develop novel NF-κB inhibitory agents, herein we reported the synthesis of various amide, amine derivatives of 3,4-dihydro-2*H*-benzo[*h*]chromene and their structure-activity relationship (SAR).

As shown in **Scheme 1**, we commenced our synthesis with commercially available **5** which involved in tandem cyclization with diethyl oxalate, sodium ethoxide and ethanol in acidic medium provided **6**. The reduction of **6** with Pd/C obtained **7**. Hydrolysis of ester **7** which is on treatment with KOH in aqueous isopropyl alcohol was accomplished to acid **8** in 50% overall yield. After obtaining acid **8**, we furnished amidation reaction with various substituted anilines **9** using coupling reagent such as 1,1'-carbonyldiimidazole (CDI) in tetrahydrofuran (THF) afforded eleven compounds of *N*-aryl-3,4-dihydro-2*H*-benzo[*h*]chromene-2-carboxamide derivatives (**1a-k**). After synthesis of these derivatives we noticed that in evaluation of inhibitory studies some of these derivatives have low solubility so that to overcome the obstacle we modified the amide functionality to amine functionality without disturbing the core structure of 3,4-dihydro-2*H*-benzo[*h*]chromene as depicted in **Scheme 2**. To introduce the amine functionality to the core of 3,4-dihydro-2*H*-benzo[*h*]chromene we initiated our synthetic plan from acid **8** which is on reduction with lithium aluminum hydride ($LiAlH_4$) in tetrahydrofuran (THF) afforded 99% of alcohol **10** which is good precursor for the synthesis of aryl amine and alkyl amine derivatives of 3,4-dihydro-2*H*-benzo[*h*]chromene as shown in **scheme 2**. Alcohol **10** on treatment with 3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and triphenylphosphine ($PPh_3$) in acetonitrile at reflux temperature obtained six *N*-((3,4-dihydro-2*H*-benzo[*h*]chromen-2-yl)methyl)-2-methylaniline derivatives **2a-f** as depicted in **scheme 2**. We have also synthesized various 3,4-dihydro-2*H*-benzo[*h*]chromene derivatives **3a-d** and **4a-d** from the precursor **10** which is at first involved in tosylation reaction with TsCl/DMAP/$Et_3N$ in DCM at 0°C provided 76% of tosylated product which is also converted to azide functionality in 97% yield by treatment with $NaN_3$/DMF at 60° C. Further the azide compound involved in Staudinger reaction[18] with triphenylphosphine ($PPh_3$) in tetrahydrofuran (THF) obtained 88% of **11** as shown in **Scheme 2**. We have derived four methyl amide derivatives **3a-d** and four alkyl amine derivatives **4a-d** from **11** as depicted in **Scheme 2**.




**Scheme 2.** General methods to prepare 2,3 & 4 series. Reagent and condition: (f) LAH, THF, reflux, 3 h, 99% (g) DDQ, $PPh_3$, MeCN, reflux, 12 h, 9-22% (h) DMAP, $Et_3N$, TsCl, $CH_2Cl_2$, 0 °C to rt, 3 h, 76% (i) $NaN_3$, DMF, 60 °C, 97% (j) TPP, $H_2O$, THF, rt, 88% (k) $Et_3N$, THF, 0 °C to rt, 30 min, 34-91%, (l) LAH, THF, 3 h, 32-42%

**Table 1.** Inhibitory effect on LPS-induced NF-κB transcriptional activity for 3,4-dihydro-2*H*-benzo[*h*]chromene derivatives.

| NO. | Substituents (R group) | | | | % inhibition at 100 μM | $IC_{50}$[a] (μM) | NO. | Substituents (R group) | | | | % inhibition at 100 μM | $IC_{50}$[a] (μM) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | | | | $R_1$ | $R_2$ | $R_3$ | $R_4$ | | |
| **PDTC** | Ref | | | | | 37.2 | **2a** | H | H | H | H | 49 | - |
| **KL-1156** | Ref | | | | | 40.1 | **2b** | H | H | $OCH_3$ | H | 88 | 44.5 |
| **1a** | H | H | H | H | 62 | 43.5 | **2c** | H | H | Cl | H | 94 | 26.5 |
| **1b** | OH | H | H | H | 91 | 12.3 | **2d** | H | Cl | H | H | 36 | - |
| **1c** | H | H | OH | H | >100 | 5.0 | **2e** | H | H | $CF_3$ | H | 42 | - |
| **1d** | H | H | $OCH_3$ | H | >100 | 12.9 | **2f** | H | $CF_3$ | H | $CF_3$ | 96 | 56.3 |
| **1e** | H | H | Cl | H | >100 | 5.8 | **3a** | phenyl | | | | >100 | 48.6 |
| **1f** | H | Cl | H | H | 87 | 27.9 | **3b** | methyl | | | | 35 | - |
| **1g** | H | Cl | Cl | H | 40 | - | **3c** | isopropyl | | | | 31 | - |
| **1h** | H | Cl | H | Cl | 52 | 96.7 | **3d** | isobutyl | | | | 77 | 56.3 |
| **1i** | $CF_3$ | H | H | H | >100 | 2.4 | **4a** | phenyl | | | | >100 | 14.6 |
| **1j** | H | H | $CF_3$ | H | >100 | 12.9 | **4b** | methyl | | | | 39 | - |
| **1k** | H | $CF_3$ | H | $CF_3$ | 66 | 63.8 | **4c** | isopropyl | | | | >100 | 27.1 |
| | | | | | | | **4d** | isobutyl | | | | >100 | 25.9 |

[a] $IC_{50}$ values are taken as a mean from three experiments and exhibiting 50% inhibition of LPS-induced NF-κB transcriptional activity.

As shown in **Table 1**, a total twenty five compounds of 3,4-dihydro-2*H*-benzo[*h*]chromene derivatives were evaluated for inhibitory effect on LPS-induced NF-κB transcriptional activity[19] and also compared to parent lead compound KL-1156 and reference compound pyrrolidine dithiocarbamate (PDTC), which acts as an antioxidant and potent inhibitor of NF-κB activation.[20] Among all evaluated twenty five compounds when compared to **KL-1156** ($IC_{50}$: 40.1 μM) and **PDTC** ($IC_{50}$: 37.2 μM); **1b** ($IC_{50}$: 12.3 μM), **1c** ($IC_{50}$: 5.0 μM), **1d** ($IC_{50}$: 12.9 μM), **1e** ($IC_{50}$: 5.8 μM), **1f** ($IC_{50}$: 27.9 μM), **1i** ($IC_{50}$: 2.4 μM), **1j** ($IC_{50}$: 12.9 μM), **2c** ($IC_{50}$: 26.5 μM), **4a** ($IC_{50}$: 14.6 μM), **4c** ($IC_{50}$: 27.1 μM) and **4d** ($IC_{50}$: 25.9 μM), exhibited more good potent activity. In particular *N*-(2-(trifluoromethyl)phenyl)-3,4-dihydro-2*H*-benzo[*h*]chromene-2-carboxamide **1i** ($IC_{50}$: 2.4 μM) exhibited excellent inhibitory activity among all synthesized 3,4-dihydro-2*H*-benzo[*h*]chromene derivatives (**1a-k**) and it was found to be about 16 and 15 times more potent than parent lead and reference compounds **KL-1156** and **PDTC** respectively. Not only above compounds but also **1c** ($IC_{50}$: 5.0 μM) and **1e** ($IC_{50}$: 5.8 μM) also exhibited better inhibitory activities than **KL-1156** and **PDTC** as shown in **Table 1**. From Table 1, we concluded that **1a-k** derivatives exhibited outstanding inhibitory activities than **2a-f** , **3a-d** and **4a-d** derivatives when evaluated in LPS-stimulated macrophage RAW 264.7 cells.

To explore the structure-activity relationship of *N*-(substituted phenyl)-3,4-dihydro-2H-benzo[*h*]chromene-2-carboxamide derivatives (**1a-k**) toward NF-κB inhibition, we substituted different groups at planar phenyl ring and also been observed that the substitutions at *ortho* ($R_1$ substitution) and *para* ($R_3$ substitution) positions of phenyl ring exhibited good inhibitory activities than compared to *meta* ($R_4$ substitution) in evaluation of LPS-stimulated macrophage RAW 264.7 as shown in **Table 1**. For instance *ortho* and *para* substitution analogs **1b**, **1i**, **1d**, **1e**, **1j** exhibited good inhibitory activities than *meta* substitution analogs **1f**, **1h**, **1k**. But in the case of **4a-d** derivatives we could not find any structure-activity relationship (SAR) since they have also exhibited better inhibitory activity of NF-κB. Among them bulky substitutions benzyl, isobutyl, isopropyl containing analogs **4a** ($IC_{50}$: 14.6 μM), **4c** ($IC_{50}$: 27.1 μM) and **4d** ($IC_{50}$: 25.9 μM) respectively exhibited better activity than parent lead compound KL-1156 and reference PDTC as depicted in **Table 1**.

**Table 2.** In vitro anti-proliferative activity against NCI-H23 cancer cell lines[21]

| No. | [a]$GI_{50}$ (μM) | No. | [a]$GI_{50}$ (μM) | No. | [a]$GI_{50}$ (μM) |
|---|---|---|---|---|---|
| | NCI-H23 | | NCI-H23 | | NCI-H23 |
| **ADR** | 0.178 | **1h** | >30 | **2f** | 14.62 |
| **KL-1156** | 9.316 | **1i** | 0.521 | **3a** | 11.38 |
| **1a** | >30 | **1j** | 3.482 | **3b** | >30 |
| **1b** | >30 | **1k** | 11.18 | **3c** | >30 |
| **1c** | 11.28 | **2a** | 25.78 | **3d** | >30 |
| **1d** | 3.977 | **2b** | 12.77 | **4a** | 10.43 |
| **1e** | 2.794 | **2c** | 29.76 | **4b** | >30 |
| **1f** | >30 | **2d** | 17.17 | **4c** | 11.72 |
| **1g** | 23.47 | **2e** | >30 | **4d** | 11.3 |

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

For finding the correlation between the inhibitory activities of NF-κB and anti-proliferative activities (cytotoxicity) a total twenty-five compounds were also evaluated for anti-proliferative activity against NCI-H23 human lung cancer cell line (**Table 2**). The results of anti-proliferative activity in NCI-H23 cell line was not directly proportional to NF-κB inhibitory activities. However, compounds exhibiting good inhibitory activities of NF-κB also exhibited better anti-proliferative activities in NCI-H23 cell line. Among them interestingly **1i** exhibited the outstanding NF-κB inhibitory activity as well as excellent anti-proliferative activity than other compounds against NCI-H23 cell line and which is also found to have comparable anti-proliferative activity of doxorubicin (**ADR**) ($GI_{50}$: 0.178 μM). From the **Table 2**, we concluded that the **1a-k** derivatives exhibited more potent activities and maintained a

good correlation between their NF-κB inhibition and anti-proliferative activities than **2a-f**, **3a-d** and **4a-d** derivatives in NCI-H23 lung cancer cell line.

In conclusion, a total twenty-five compounds of 3,4-dihydro-2*H*-benzo[*h*]chromene have been synthesized and evaluated their inhibitory effects on LPS-induced NF-κB transcriptional activity. Among them **1a-k** derivatives exhibited good inhibitory activities than **2a-f**, **3a-k** and **4a-g** series. Structure-activity relationship of **1a-k** analogs appears to be essential for its NF-κB transcriptional activities and slight substitution variation at *ortho* position ($R_1$) like **1i**[22] ($IC_{50}$: 2.4 μM) surprisingly exhibited excellent inhibitory activity on LPS-induced NF-κB transcriptional activity than parent lead compound KL-1156 and reference compound PDTC and it is also exhibiting good anti-proliferative activity than KL-1156. This might indicate that 3,4-dihydro-2*H*-benzo[*h*]chromene core could be the lead scaffold for investigating new anticancer agent through inactivation of NF-κB.

## Acknowledgments

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## Supplementary data

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

## References and notes


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19. *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.
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21. *In vitro anti-proliferative activity (cytotoxicity) assay* was performed using the number of cells was measured indirectly using the sulforhodamine B (SRB) method according to the NCI (USA) protocol (see ref. 13a). Briefly, the cells were plated into a 96 well plate at a density of 2 × 103 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 - 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 one 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.
22. *N-(2-(trifluoromethyl)phenyl)-3,4-dihydro-2H-benzo[h]chromene-2-carboxamide (**1i**):* IR (neat): 3307, 2946, 1661, 1320, 1023, 652 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): $\delta$ 9.10 (s, 1H, NH), 8.42 (d, $J$ = 8.3 Hz, 1H, Ar-H), 8.23 (d, $J$ = 8.3 Hz, 1H, Ar-H), 7.79 (d, $J$ = 7.4 Hz, 1H, Ar-H), 7.65 - 7.47 (m, 5H, Ar-H), 7.26 (t, $J$ = 7.6 Hz, 1H, Ar-H), 7.19 (d, $J$ = 8.4 Hz, 1H, Ar-H), 4.85 (dd, $J$ =9.9 Hz, 2.9 Hz, 1H, $OCHCH_2CH_2$), 3.14 - 2.93 (m, 2H, $OCHCH_2CH_2$), 2.67 - 2.60 (m, 1H, $OCHCH_2CH_2$), 2.31 - 2.21 (m, 1H, $OCHCH_2CH_2$). $^{13}C$ NMR ($CDCl_3$, 100 MHz): $\delta$ 169.6, 146.9, 138.4, 133.4, 132.7, 132.4, 128.0, 127.5, 126.2, 126.1, 124.6, 124.4, 121.5, 120.2, 119.8, 119.8, 118.1, 116.4, 75.8, 24.9, 24.0. HRMS Calcd. For $C_{21}H_{16}F_3NO$ *m/z* 371.11 found $[M+Na]^+$ 394.27.

# Design and Synthesis of 3,4-Dihydro-2*H*-benzo[*h*]chromene Derivatives as Potential NF-κB Inhibitors

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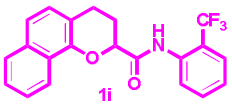


1i

$IC_{50}$: 2.4 μM