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Structure–activity relationship of indoline-2-carboxylic acid *N*-(substituted)phenylamide derivatives

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ABSTRACT

Chroman derivatives exhibited potent inhibitory activity of NF-κB. For SAR, the chroman scaffold was modified with an indoline moiety. A series of indoline-2-carboxylic acid *N*-(substituted)phenylamide derivatives were synthesized to explore their inhibitory activities of NF-κB and they were also evaluated for cytotoxicity against various cancer cell lines. Since intermediates with Boc showed outstanding results, various substituents in place of the Boc group were introduced additionally and these compounds were also evaluated for SAR.

Nuclear factor-κB, a multifunctional transcription factor, plays critical roles in physiological and pathological processes such as immune function, cellular growth, homeostasis, apoptosis, and inflammation. NF-κB consists of heterodimers or homodimers formed by members of the NF-κB family, P65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2), in mammalian cells. In the cytoplasm, NF-κB dimer exists in an inactive state and is associated with IκB proteins that mask the nuclear localization signal (NLS) in NF-κB protein.[1,2]

There are two major pathways leading to activation of NF-κB, the canonical (classic) pathway and non-canonical (non-classic) pathway. The canonical pathway, the major pathway, consists of IκB kinase (IKK), IκB, and NF-κB (typically p65/p50 heterodimer). IKK is activated by extracellular signals such as tumor necrosis factor α (TNF-α), interlukin 1 (IL-1), lipopolysaccharide (LPS), reactive oxygen species (ROS), and a variety of cellular stresses. The activated IKK phosphorylates the serine residues at positions 32 and 36 in IκB. The phosphorylation further causes ubiquitination of IκB, and then induces proteasome degradation of IκB. The activated NF-κB by exposure of the NLS translocates to the nucleus, where it transcribes genes.[3–6]

The non-canonical pathway involves NF-κB inducing kinase (NIK), IKKα, and a p100/RelB heterodimer complex. p100/RelB is phosphorylated by NIK mediated activation of IKKα and partially degraded to p52/RelB through a ubiquitin-dependent mechanism. The p52/RelB complex translocates to the nucleus to enhance gene expression.[3]

Irregular gene expression though NF-κB is responsible for inflammation, autoimmune diseases, viral infections, and various cancers. Therefore, it has been suggested that inhibitors of NF-κB function may be useful both as anti-inflammatory agents and as antitumor agents.[2,7] We recently reported a multiplicity of chroman derivatives having inhibitory activity of NF-κB.[8–11] They were designed based on compound **KL-1156** (6-hydroxy-7-methoxychroman-2-carboxylic acid phenylamide), an inhibitor of translocation to the nucleus in LPS-stimulated macrophage RAW 264.7 cells.[8] In previous studies it was found that the phenolic OH group of the chroman moiety was not required for enhancement of NF-κB inhibitory activity.[10] In chroman derivatives with a short alkyl group, there are no significant differences between substituent effects and the inhibition potency.[11]

To establish a systematic SAR of pyranyl moiety in chroman compounds, introduction of nitrogen atom together with ring contraction, could be considered. Furthermore, an indoline scaffold is recognized as a privileged pharmacophore,[12] 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, we replaced the chroman scaffold with the indoline moiety. Substituents (H, OH, OCH_3, CH_3, NO_2, Cl, and CF_3) on the

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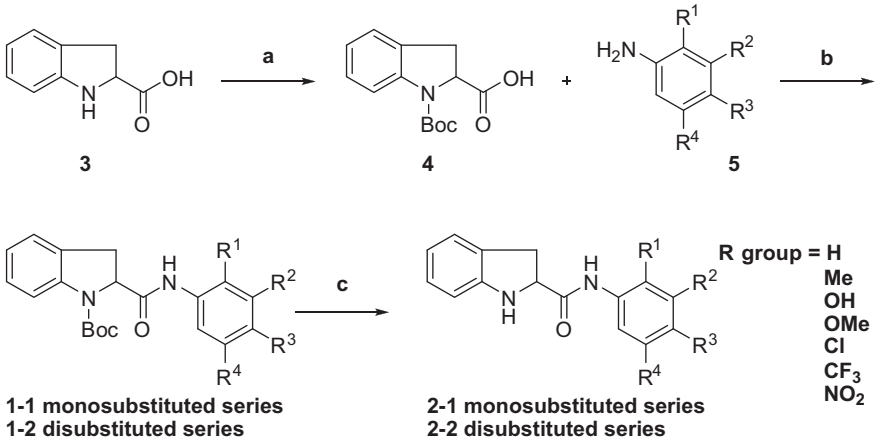
N-phenyl ring moiety of target compounds were selected according to their electronic and hydrophobic character. The positional effects of the substituents were also explored by examining compounds with substituents at various positions (2-; 3-; 4-; 3,4-; 3,5-; 2,5-) on the phenyl ring. Herein, we report the syntheses and structure–activity relationship (SAR).

The indoline-2-carboxylic acid *N*-(substituted)phenyl amide derivatives were prepared starting from the commercially available indoline-2-carboxylic acid as depicted in Scheme 1. The indoline-2-carboxylic acid was protected with di-*tert*-butyl dicarbonate ($(Boc)_2O$) to give *N*-Boc-indoline-2-carboxylic acid. Amidation between acid **4** and substituted phenylamines **5** using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in tetrahydrofuran (THF) was followed by removal of the Boc protecting group with trifluoroacetic acid (TFA) in dichloromethane to afford the indoline **2** series. A total of twenty-nine compounds of indoline derivatives were synthesized and evaluated in terms of their inhibitory activity of NF-κB. In addition, intermediates, *N*-Boc-indoline derivatives, were evaluated in LPS-stimulated macrophage RAW 264.7 cells (Table 1).

One compound of indoline-2-carboxamide derivatives (**2**–**2j**; IC_{50}: 12.0 μM) and six compounds (**1**–**1l**, **1**–**1p**, **1**–**2a**, **1**–**2b**, **1**–**2e**, and **1**–**2j**) of *N*-Boc-indoline-2-carboxamide derivatives (IC_{50}: 10.1–16.6 μM) exhibited more potent activity than KL-1156 (IC_{50}: 40.1 μM). *N*-Boc-indoline-2-carboxylic acid *N*-(3,5-dichlorophenyl) amide (**1**–**2b**; IC_{50}: 10.1 μM) showed the best activity. The positional effects of substituents of the indoline moiety on inhibitory activities are contradictory. Direct correlation was not



Scheme 1. General methods to prepare **2** series. Reagents and conditions: (a) Boc_2O, NaOH, dioxane, H_2O, 2 h, 95%; (b) EDC, THF, 14 h, 40–98%; (c) TFA, DCM, 6 h, 25–98%.

Table 1
Inhibitory effect on LPS-induced NF-κB transcriptional activity for intermediate **1** series and indoline derivative **2** series

Substituents (R group on phenyl ring)				No.	**1** series		No.	**2** series	
R^1	R^2	R^3	R^4		% inhibition at 100 μM	IC_{50} (μM)		% inhibition at 100 μM	IC_{50} (μM)
Ref.				**KL-1156**	90 ± 8	40.1	**KL-1156**	90 ± 8	40.1
H	H	H	H	**1–1a**	30 ± 5	>100	**2–1a**	65 ± 6	71.6
OH	H	H	H	**1–1b**	93 ± 1	—	**2–1b**	37 ± 5	>100
H	OH	H	H	**1–1c**	75 ± 8	58.7	**2–1c**	30 ± 5	>100
H	H	OH	H	**1–1d**	42 ± 4	>100	**2–1d**	63 ± 3	66.2
OCH_3	H	H	H	**1–1e**	76 ± 2	—	**2–1e**	60 ± 5	79.2
H	OCH_3	H	H	**1–1f**	38 ± 3	>100	**2–1f**	64 ± 3	—
H	H	OCH_3	H	**1–1g**	29 ± 3	>100	**2–1g**	37 ± 9	>100
CH_3	H	H	H	**1–1h**	68 ± 1	62.5	**2–1h**	39 ± 5	>100
H	CH_3	H	H	**1–1i**	87 ± 2	—	**2–1i**	29 ± 3	>100
H	H	CH_3	H	**1–1j**	37 ± 6	>100	**2–1j**	41 ± 8	>100
NO_2	H	H	H	**1–1k**	63 ± 1	—	**2–1k**	41 ± 6	>100
H	NO_2	H	H	**1–1l**	94 ± 2	14.1	**2–1l**	49 ± 5	>100
H	H	NO_2	H	**1–1m**	>100	—	**2–1m**	41 ± 4	>100
CF_3	H	H	H	**1–1n**	38 ± 4	>100	**2–1n**	61 ± 2	79.4
H	CF_3	H	H	**1–1o**	>100	—	**2–1o**	99 ± 1	46
H	H	CF_3	H	**1–1p**	>100	12	**2–1p**	56 ± 5	79.2
Cl	H	H	H	**1–1q**	50 ± 1	—	**2–1q**	62 ± 10	69.7
H	Cl	H	H	**1–1r**	>100	—	**2–1r**	63 ± 3	—
H	H	Cl	H	**1–1s**	31 ± 3	>100	**2–1s**	62 ± 4	70.7
H	Cl	Cl	H	**1–2a**	97 ± 3	12.6	**2–2a**	72 ± 3	—
H	Cl	H	Cl	**1–2b**	—	10.1	**2–2b**	98 ± 3	—
Cl	H	H	Cl	**1–2c**	71 ± 4	45.9	**2–2c**	34 ± 9	>100
H	CH_3	CH_3	H	**1–2d**	64 ± 3	—	**2–2d**	87 ± 2	43.3
H	CH_3	H	CH_3	**1–2e**	78 ± 9	16.6	**2–2e**	56 ± 3	85
CH_3	H	H	CH_3	**1–2f**	75 ± 4	50	**2–2f**	47 ± 7	>100
H	OCH_3	OCH_3	H	**1–2g**	65 ± 1	52.2	**2–2g**	15 ± 8	>100
H	OCH_3	H	OCH_3	**1–2h**	88 ± 1	—	**2–2h**	41 ± 4	>100
OCH_3	H	H	OCH_3	**1–2i**	76 ± 1	—	**2–2i**	47 ± 2	>100
H	CF_3	H	CF_3	**1–2j**	—	14.9	**2–2j**	96 ± 3	12

IC_{50} values are means of the concentration (μM) exhibiting 50% inhibition of LPS-induced NF-κB transcriptional activity.

observed based on the electronic and hydrophobic character of the substituents. The most active compound, **1**–**2b**, was found to be four times more potent than the reference compound, **KL-1156**.

NF-κB is an important factor not only for cell survival signals in most cell types but also for cancer development in various organs. Incorrect regulation of NF-κB has been related to antitumor activity.[13] For the correlation between the inhibitory activity of NF-κB and cytotoxic activity, fifty-eight compounds were evaluated for cytotoxicity against NCI-H23 (lung) and PC-3 (prostate) cancer cell lines (Table 2).

Only eleven compounds showed significant cytotoxicity. Generally, compounds having NF-κB inhibitory activity exhibited predominant cytotoxicity. However, cytotoxicity was not directly proportional to NF-κB inhibitory activity. Regardless of the substituent at the nitrogen of the indoline moiety, compounds containing 3-CF_3, 3,4-diCl, 3,5-diCl or 3,5-diCF_3 on the phenyl ring exhibited significant cytotoxicity. In particular, compounds bearing 3,5-diCF_3 on the phenyl ring (**1**–**2j** and **2**–**2j**; NCI-H23: 1.524 and 0.648 μM; PC-3: 0.838 and 0.794 μM) were found to have comparable cytotoxic activity to doxorubicin (ADR, NCI-H23: 0.178 μM; PC-3: 0.317 μM).

Since intermediates with Boc showed outstanding inhibitory activity of NF-κB, especially compounds bearing 4-CF_3 (**1**–**1p**, IC_{50}: 12.0 μM) and 3,5-diCl (**1**–**2b**, IC_{50}: 10.1 μM) on the phenyl ring, various substituents (acetyl, benzoyl, 2-(Boc)amino-1-oxypropyl, nonanoyl, pivaloyl, and tosyl) instead of the Boc group were introduced on indoline-2-carboxylic acid *N*-(4-CF_3- and 3,5-diCl-)phenylamide. **6** series and **7** series compounds were prepared from compounds **1**–**1p** and **2**–**2a** (Scheme 2). These compounds were evaluated in LPS-stimulated macrophage RAW 264.7 cells for inhibitory activity of NF-κB.

In compounds with the 4-CF_3 substituent on the phenyl ring, compounds with pivaloyl (**6c**), benzoyl (**6d**), and 2-(Boc)amino-1-oxypropyl (**6e**) at the nitrogen on indoline show inhibitory activity of NF-κB similar to that of the reference compound with Boc group (**1**–**2b**). Except for the compound bearing the tosyl group (**7f**), compounds (**7a**–**e**) in the **7** series exhibited inhibitory activity of NF-κB. In particular, compounds with 2-(Boc)amino-1-oxypropyl on indoline exhibited more potent inhibitory activity than compounds bearing other substituents. The compounds **6e** and **7e** were found to be slightly more potent than the reference compound with the Boc group (**1**–**2b**) (Table 3).

The **6** and **7** series compounds were also examined for cytotoxic activity against ACHN (renal), HCT15 (colon), MM231 (breast), NUGC-3 (gastric), NCI-H23, and PC-3 cancer cell lines (Table 4). Generally, compounds having inhibitory activity of NF-κB show cytotoxicity in various cancer cell lines, as stated earlier. Poor inhibitory activity of NF-κB was related to inferior cytotoxic activity. However, compound **6b** is an exception, exhibiting cytotoxicity against ACHN, HCT15, NUGC-3, NCI-H23, and PC-3 cancer cell lines. Cytotoxicity related inhibitory activity of NF-κB varied according to cancer cell type. Compound **7b** exhibited more potent activity than other compounds against all cancer cell lines tested.

Table 2
Cytotoxicity against NCI-H23 and PC-3 cancer cell lines

	GI_{50} (μM)	
	NCI-H23	PC-3
ADR	0.178	0.317
KL-1156	9.316	—
1–**1m**	2.523	2.794
1–**1o**	6.343	3.337
1–**1p**	3.207	2.020
1–**1r**	3.230	—
1–**2a**	3.188	4.663
1–**2b**	3.390	1.676
1–**2j**	1.524	0.838
2–**1o**	4.960	4.244
2–**2a**	11.604	—
2–**2b**	12.294	2.534
2–**2j**	0.648	0.794

The GI_{50} values correspond to the agent's concentration causing a 50% decrease in net cell growth.

Table 3
Inhibitory effect on LPS-induced NFκB transcriptional activity

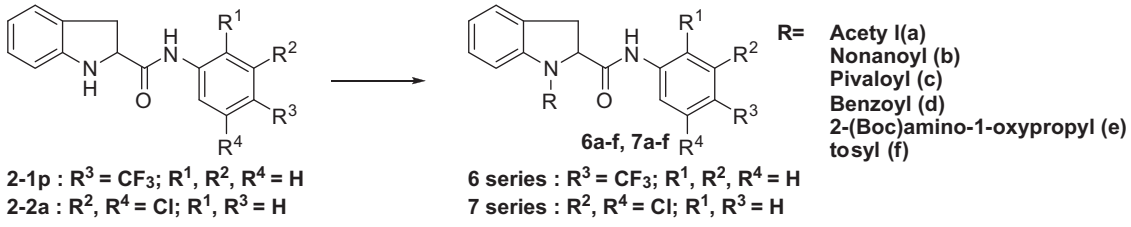
R group	4-CF_3	IC_{50} (μM)	3,5-diCl	IC_{50} (μM)
Acetyl	**6a**	>100	**7a**	34.1
Nonanoyl	**6b**	>100	**7b**	12.6
Pivaloyl	**6c**	12.7	**7c**	14.5
Benzoyl	**6d**	13.5	**7d**	46.5
2-(Boc)amino-1-oxypropyl	**6e**	7.0	**7e**	5.9
Tosyl	**6f**	>100	**7f**	>100
Ref.	—	—	**1**–**2b**	10.1

The IC_{50} values are means of the concentration (μM) exhibiting 50% inhibition of LPS-induced NF-κB transcriptional activity.

Table 4
Cytotoxicity against ACHN, HCT15, MM231, NUGC-3, NCI-H23, and PC-3 cancer cell lines

	GI_{50} (μM)					
	ACHN	HCT15	MM231	NUGC-3	NCI-H23	PC-3
ADR	0.182	2.177	0.194	0.202	0.178	0.317
6a	>10	>10	>10	>10	>10	>10
7a	>10	8.676	>10	7.856	7.678	6.37
6b	3.781	2.067	>10	3.452	1.619	1.861
7b	1.892	1.73	3.573	1.515	1.283	1.611
6c	5.627	5.535	7.311	6.581	3.645	3.885
7c	3.68	3.808	4.973	3.348	3.463	4.179
6d	—	5.491	—	8.721	4.580	—
7d	8.26	—	6.417	7.974	4.284	6.842
6e	6.992	6.512	>10	7.842	—	6.255
7e	5.037	4.326	—	6.206	6.712	4.427
6f	>10	>10	>10	>10	8.968	6.678
7f	>10	>10	>10	>10	7.534	>10

The GI_{50} values correspond to the agent's concentration causing 50% decrease in net cell growth.



Scheme 2. General methods to prepare **6** and **7** series. Reagents and conditions: (a) Ac_2O, THF, 85–90%; (b) nonanoyl chloride, THF, TEA, 60–70%; (c) PivCl, THF, TEA, 55–68%; (d) Bz_2O, THF, 40–57%; (e) Boc-alanin, EDC, THF, 43–50%; (f) TsCl, TEA, THF, 55–60%.

In summary, we have described seventy-two indoline-2-carboxamide derivatives in relation to NF-κB activity. At concentrations below 20 μM, one indoline derivative and twelve compounds with substituents at the nitrogen of the indoline scaffold showed significant activity. Compound **7e** showed the highest activity among all the indoline derivatives. The 2-(Boc)amino-1-oxypropyl group at the nitrogen of the indoline moiety slightly enhanced the inhibitory activity of NF-κB. In the results of cytotoxicity, compounds **2**–**2j** and **7b** displayed more potent cytotoxic activity than the other compounds. Compounds having inhibitory activity of NF-κB also showed cytotoxic activity in various cancer cell lines. However, cytotoxicity was not directly proportional to NF-κB inhibitory activity. Work is in progress to design, synthesize, and evaluate additional compounds in the present system and related systems.

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References and notes

1. Gilmore, T. D. *Oncogene* **1999**, *18*, 6842.
2. Hayden, M. S.; Ghosh, S. *Gene Dev.* **2004**, *18*, 2195.
3. Gilmore, T. D. *Oncogene* **2006**, *25*, 6680.
4. Israel, A.; Le Bail, O.; Hatat, D.; Piette, J.; Kieran, M.; Logeat, F.; Wallach, D.; Fellous, M.; Kourilsky, P. *EMBO J.* **1989**, *8*, 3793.
5. Osborn, L.; Kunkel, S.; Nabel, G. J. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 2336.
6. Michiels, C.; Minet, E.; Mottet, D.; Raes, M. *Free Radical Biol. Med.* **2002**, *33*, 1231.
7. Perkins, N. D.; Gilmore, T. D. *Cell Death Differ.* **2006**, *13*, 759.
8. 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.
9. Kwak, J. H.; Kim, B. H.; Jung, J. K.; Kim, Y.; Cho, J.; Lee, H. *Arch. Pharm. Res.* **2007**, *30*, 1210.
10. 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.
11. 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.
12. (a) Gan, Z.; Reddy, P. T.; Quevillon, S.; Couve-Bonnaire, S.; Arya, P. *Angew. Chem., Int. Ed.* **2005**, *44*, 1366; (b) Poondra, R. R.; Kumar, N. N.; Bijian, K.; Prakesch, M.; Campagna-Slater, V.; Reayi, A.; Reddy, P. T.; Choudhry, A.; Barnes, M. L.; Leek, D. M.; Daroszewska, M.; Lougheed, C.; Xu, B.; Schapira, M.; Alaoui-Jamili, M. A.; Arya, P. *J. Comb. Chem.* **2009**, *11*, 303.
13. Sarkar, F. H.; Li, Y.; Wang, Z.; Kong, D. *Int. Rev. Immunol.* **2008**, *27*, 293.