Synthesis and Biological Evaluation of 2-Phenylimino-5((5-phenylfuran-2-yl)methylene)thiazolidin-4-ones as IKK2 Inhibitors

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In a search for novel molecules to treat inflammatory disorders, we identified several compounds with inhibitory action against the IKK2 enzyme using *in silico* methods. Based on the virtual hit of compounds **1** and **2**, a novel series of 2-phenylimino-5((5-phenylfuran-2-yl)methylene)thiazolidin-4-one derivatives was designed, synthesized, and evaluated for IKK2 inhibitory activity. Among the synthesized derivatives, compounds **17f** and **19f** showed good IKK2 inhibitory potency, which have 4-carboxaminophenyl on the 2-furan ring and a methoxy group on the phenylimino moiety at the 2-position of the core structure. The most potent compound was 2-(2,4-dimethoxyphenyl)imino-5((5(4-carboxaminophenyl)furan-2-yl)methylene)thiazolidin-4-one (**19f**, IC_{50} = 0.94 μM), which represents a synergic effect of the two virtual hit compounds against IKK2. We also identified compounds showing inhibitory activities against interleukin (IL)-17, CCK-8, and tumor necrosis factor-alpha (TNF-α), which are NF-κB-dependent pro-inflammatory cytokine mediators.

Keywords: 2-Phenylimino-5((5-phenylfuran-2-yl)methylene)thiazolidin-4-one, IKK2 inhibitor, Anti-inflammatory activity

Introduction

The IκB kinase IKK2, also designated IKKβ, activates members of the NF-κB transcription factor via the canonical pathway by phosphorylation of IκB kinase inhibitors.[1] The transcriptional nuclear factor κ-B (NF-κB) is a key factor in the transcription of numerous inflammatory genes. NF-κB plays a crucial role in immunological function in the pathogenesis of various diseases including autoimmune disorders, asthma, rheumatoid arthritis, cancer, and diabetes.[2] The heteromeric or homomeric dimer complex binds to specific inhibitors of NF-κB, known as IκB proteins.[3] The IKK complex is composed of three subunits: the catalytic subunits IKKα (IKK1) and IKK-β (IKK2) and the regulatory IKK-γ (NEMO) subunit.[4] NF-κB activation functions through two pathways: canonical and non-canonical. In the canonical pathway, signaling is induced by the IKK complex, and active NF-κB subsequently translocates into the nucleus and activates the transcription of pro-inflammatory cytokines. In the non-canonical pathway, NF-κB activation can be initiated by IKK1 homodimers.[5] Although the mechanism of regulation remains poorly understood, complex target validation of NF-κB signaling, IκB kinase (IKK1 and IKK2), plays a major role in NF-κB activation arising from pro-inflammatory stimuli such as tumor necrosis factor-alpha (TNF-α), interleukin IL-6, IL-1β, and IL-17. Moreover, although IKK1 and IKK2 have similar structural domains, IKK2 has higher kinase activity towards Iκ-B than IKK1.[6] Therefore, IKK2 is a key molecule involved in signaling to the transcription factor NF-κB and is a novel target for autoimmune and inflammatory diseases and different types of cancer by interfering with NF-κB activation.[7] In significant efforts to identify potent and selective IKK inhibitors, various types of small-molecule inhibitors have been patented, such as pyrimidines, quinazolines, thiophenecarboxamides, pyrines, fused imidazoles, pyrazoles, benzimidazoles, indoles, and carbolin derivatives.[8] In this study, we describe the structure–activity relationship (SAR) on a new scaffold as IKK2 inhibitors. Based on the hit compounds, we designed, synthesized, and evaluated novel thiazolidinone derivatives for the inhibitory activity against IKK2.

To identify novel small-molecule inhibitors of IKK2 for treating inflammatory disorders, we performed *in silico* virtual screening of home libraries and identified several hit compounds.

Based on the findings that virtual hit **1** is composed of an iminothiazolidinone core with a benzylidene functional group and hit **2** has a pyrazolidinone core with a benzylfuranylmethylene group, we designed and synthesized the novel 2-phenyllimino-5[(5-phenylfuran-2-yl)methylene] thiazolidin-4-ones **3** (Figure 1).

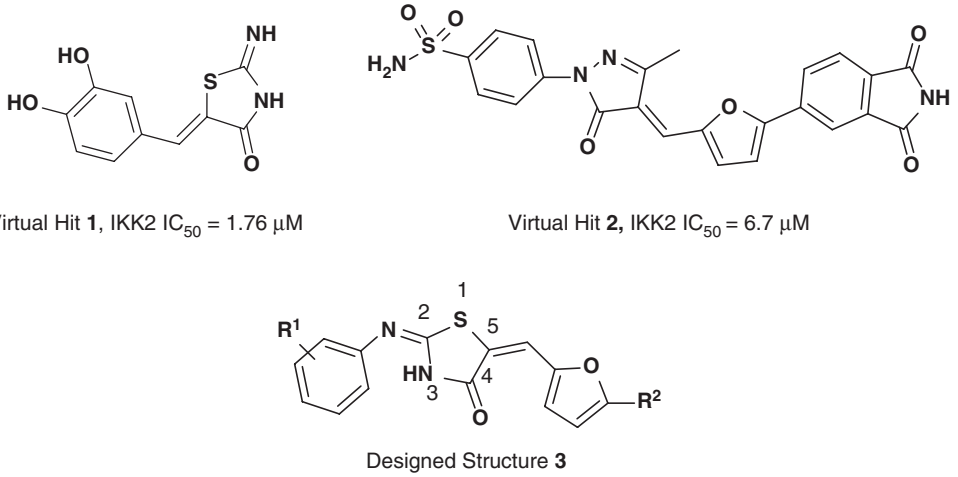


Figure 1. Structure of the virtual hit compounds **1** and **2** and the designed structure **3**.

Chemistry

Synthesis of 2-phenylimino-5((5-phenylfuran-2-yl)methylene)thiazolidin-4-ones was performed as shown in Scheme 1. The 2-(phenylimino)thiazolidin-4-ones (**6a–l**) core ring was assembled via one of the two routes outlined. Route **A** starts with treatment of various R^1 substituted anilines **4** with ammonium thiocynate under acidic conditions to afford the corresponding phenylthiourea **5**, which was then reacted with chloroethyl acetate to yield differentially substituted thiazolidin-4-ones **6**. In route **B**, commercially available 2-thioxothiazolidin-4-one **7** was treated with methyl iodide to yield 2-(methylthio)thiazolidin-4-one **8**, which was then heated at reflux with substituted aniline **4** in ethanol to yield the corresponding thiazolidinones **6**. Thus, the coupling of 2-(phenylimino)thiazolidin-4-ones **6** with substituted 5-phenylfuran-2-aldehydes **10a–l** was done from 5-bromofuran-2-aldehyde **9** through the palladium-catalyzed Suzuki–Miyamura coupling reaction,[9] followed by the thermal dehydration reaction under piperidine and ethanol conditions to afford the final compounds **11–23**.

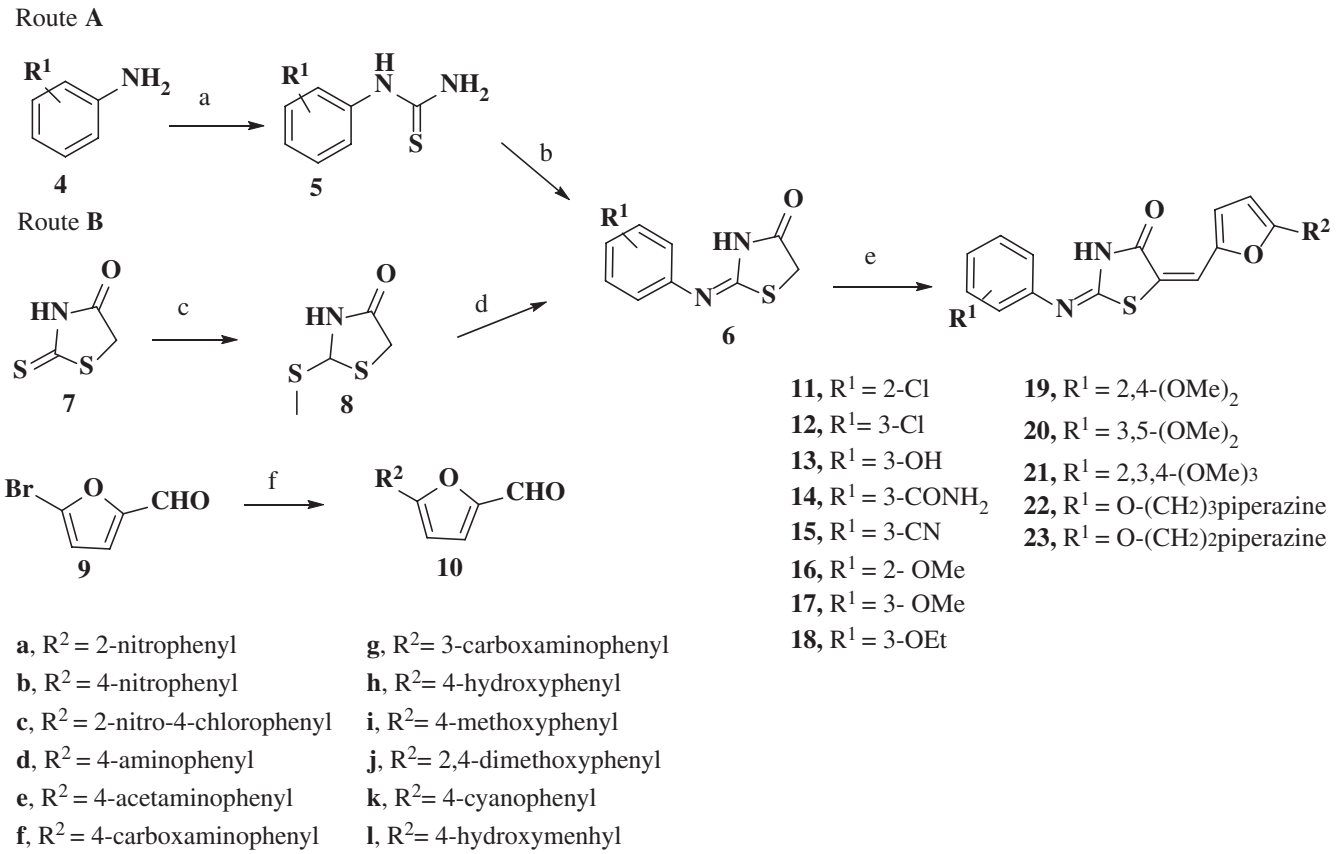
Experimental

All chemicals were reagent grade and purchased from Sigma-Aldrich. ^{1}H NMR and ^{13}C NMR spectra were recorded in δ units relative to deuterated solvent as an internal reference using a 300 or 400 MHz NMR instrument. Flash column chromatography was performed on silica gel (230–400 mesh). Mass spectra were recorded on Shimadzu Excellence in Science LCMS-2020 mass spectrometer (Kyoto, Japan) equipped with a Shimpak-VP ODS (150 mm × 2.0) column (Kyoto, Japan) using electrospray ionization and a UV detector.

General Procedure: Synthesis of Substituted Phenylthiourea (5). To a solution of substituted benzoyl chloride (8 mL, 68.73 mmol) in acetone (15 mL) was added ammonium thiocyanate (5.93 g, 77.89 mmol) at room temperature. After the reaction solution was stirred at 60 °C for 10 min, substituted aniline (45.92 mmol) in acetone (5 mL) was added slowly. The mixture was stirred at 60 °C for 1 h and then extracted with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), and filtered. The solvent was evaporated under reduced pressure, and the residual mixture was poured into diethyl ether to afford white solids. The solid was stirred in 10% sodium hydroxide solution at 80 °C for 1 h and neutralized by 4 N hydrogen chloride to yield a solid. The crude solid was filtered, washed with 50% methanol, and dried by reduced pressure to give a white solid (3,5-dimethoxyphenyl)thiourea. Yield 86%; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.71 (brs, 1H), 6.40 (d, J = 2.2 Hz, 1H), 6.37(d, J = 2.2 Hz, 2H), 6.15 (brs, 2H), 3.80 (s, 6H).

Synthesis of 2-Methylmercapto-4-thiazolidinone (8). To a solution of 2-thioxo-4-thiazolidinone (1.33 g, 10 mmole) in 2% aq. NaOH (25 mL) was added methyl iodide (1.56 g, 11 mmol), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was extracted with dichloromethane, and the organic layer was washed with brine, dried ($MgSO_4$), and filtered. The solvent was concentrated under reduced pressure to obtain a solid. 1.26 g, Yield 86%; ^{1}H NMR (300 MHz, $CDCl_3$) δ:4.01(2, 2H), 2.71 (s, 3H).

General Procedure: Preparation of 2-(Substituted phenylimino)thiazilidin-4-one (6). In route **A**, to a solution of substituted phenylthiourea (17.46 mmol) in ethanol (4 mL) was added sodium acetate (52.38 mmol), after which the reaction mixture was stirred at room temperature for 30 min. Ethyl chloroacetate (34.92 mmol) was added to the reaction solution, which was heated at reflux for 4 h. The solvent was evaporated by reduced pressure and extracted using ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$), concentrated, and the residue was purified by crystallization with diethyl ether to obtain the title compound. In route **B**, to a solution of **8** (1.47 g, 10 mmol) in ethanol 30 mL) was added substituted aniline (10 mmol), and the reaction mixture was heated at reflux overnight to give yellow solid. The solid was filtered and purified by recrystallization using ethanol to obtain the title compound 2-(3,5-dimethoxyphenylimino)



Scheme 1. Synthesis of 2-phenyllimino-5((5-phenylfuran-2-yl)methylene)thiazolidin-4-one derivatives. Reagents and conditions: (a) NH_4SCN, 6 N HCl, 80 °C; (b) 2-chloroethyl acetate, sodium acetate, ethanol, 60 °C; (c) methyl iodide, aq. 2% NaOH, rt; (d) aniline **4**, ethanol, reflux; (e) 5-R^2-furan-2-carbaldehyde **10**, piperidine, ethanol, rt; (f) 5-bromofuran-2-carboaldehyde, substituted phenylboronic acid, $(Ph_3P)_3Pd$, K_2CO_3, toluene/ethanol, 90 °C.

thiazolidin-4-one. Yield 78%; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.44 (s, 1H), 6.93 (s, 1H), 6.29 (s, 1H), 6.13 (s, 1H), 3.97 (s, 2H), 3.73 (s, 6H).

General Procedure. Synthesis of 5-(Substituted phenyl) furan-2-aldehyde (10). To a solution of substituted phenylboronic acid (3.87 mmol) in toluene/ethanol (50/50, 40 mL), 5-bromofuran-2-alsehyde (600 mg, 3.43 mmol), potassium carbonate (947.96 mg, 6.86 mmol) aq. solution (10.3 mL water), and tetrakis(triphenyphosphine)palladium(0) (35.78 mg, 0.03 mmol) were added slowly, after which the reaction mixture was stirred at 90 °C. The mixture was acidified by 6 N hydrogen chloride solution, and extracted with dichloromethane. The organic layer was washed with brine, dried ($MgSO_4$) and, filtered. The solvent was concentrated under reduced pressure to yield compound **10**, 5(4-carboxaminophenyl)furan-2-aldehyde. Yield 60%; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.65 (s, 1H), 8.11 (s, 1H), 8.02–7.94 (m, 4H), 7.69 (d, J = 3.7 Hz, 1H), 7.50 (s, 1H), 7.42 (d, J = 3.7 Hz, 1H).

General Procedure for (2-Substituted phenylimino-5 ((substituted phenyl)furan-2-yl)methylene)thiazolidin-4-one derivatives (11–23). To obtain a solution of the above substituted phenyliminothiazilidin-4-one (**6**) (0.27 mmol) in ethanol (3 mL), piperidine (27.4 μM, 0.27 mmole) and 2-(substituted phenylimino)thiazolidin-4-one (0.27 mmol) were added and stirred at 70 °C for 12 h to yield a solid. The solid was filtered and washed with diethyl ether to afford the title compound. Spectral data of selected compound 2-(3-carboxaminophenyl)imino-5((5(4-hydroxyphenyl)furan-2-yl)methylene)-thiazolidin-4-one (**14h**). Yield 45%; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.89 (s, 1H), 8.23–8.18 (q, J = 8.1Hz, 2H), 8.01 (d, J = 8.2 Hz, 1H), 7.72–7.65 (q, J = 8.9 Hz, 2H), 7.40 (d, J = 8.9 Hz, 1H), 7.29 (s, 1H), 7.23 (t, J = 8.8 Hz, 1H), 7.01 (d, J = 8.8 Hz, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.83 (s, 1H), 6.77 (s, 1H), 6.70 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 188.8, 162.9, 136.7, 141.8, 137.3, 131.5, 127.3, 125.5, 125.2, 124.7, 115.0, 114.7, 112.3, 67.6, 67.0, 65.8, 63.2, 55.9, 54.4, 26.9, 15.2.; m.p. 234–236 °C; LCMS (ESI^+) calcd. for [M + H^+] 406.1, found 406.0.

2-(3-Methoxyphenyl)imino-5((5(4-carboxaminophenyl) furan-2-yl)methylene)-thiazolidin-4-one (**17f**). Yield 92% ; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.14 (s, 1H), 7.99 (d, J = 8.0 Hz, 1H), 7.92–7.87 (m, 2H), 7.71 (d, J = 8.0 Hz, 1H), 7.58–7.33 (m, 5H), 7.56 (dd, J_1 = 22.3Hz, J_2 = 3.4 Hz, 1H), 6.86–6.71 (d, J = 5.2 Hz, 3H).; ^{13}C NMR (75 MHz, DMSO-d_6) δ:162.7, 154.4, 142.2, 134.8, 134.2, 133.1, 130.7, 129.6, 128.8, 127.4, 122.0, 121.8, 120.2, 112.2, 67.4, 60.4, 57.0, 55.2, 46.2, 30.2, 23.6.; mp 151–153 °C; LCMS (ESI^+) calcd. for [M + H^+] 420.1, found 420.0.

2-(2,4-Dimethoxyphenyl)imino-5((5(4-carboxaminophenyl)furan-2-yl)methylene)-thiazolidin-4-one (**19f**); Yield 51% ; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.03 (d, J = 8.5 Hz, 2H), 7.89 (d, J = 8.0 Hz, 1 H), 7.76 (s, 1H), 7.43 (s, 1H0, 7.46 (d, J = 8.2 Hz, 1H), 7.11 (d, J = 8.1 Hz, 2H), 6.96 (s, 1H), 6.98 (s, 1H), 6.30 (s, 1H).; ^{13}C NMR (75 MHz, DMSO-d_6) δ: 167.6, 162.3, 161.5, 161.5, 161.1, 155.3, 150.6, 134.3, 131.9, 128.8, 128.7, 124.0, 119.9, 116.6, 115.6, 111.6, 100.3, 99.6, 97.5, 96.9, 55.8. m.p. 229 −230°C; LCMS (ESI$^+$) calcd. for [M + H$^+$] 450.1, found 450.0.

Time-resolved Fluorescence Resonance Energy Transfer (TR−FRET) Assay for IKK2. IKK2 kinase reactions were performed in a reaction buffer (10 mM Tris−HCl, pH 7.2, 10 mM $MgCl_2$, 0.05% NaN_3) containing 1 mM DTT and 0.01% Tween-20 (Sigma-Aldrich) to stabilize the enzyme. The reactions were performed at room temperature for 2 h in white standard 384 plates (3572, Corning Life Sciences, Lowell, MA, USA) using 0.5 μg/mL IKK2 (Millipore Co., Billerica, MA, USA), 1 μM IκBα-derived substrate (5FAMGRHDSGLDSMK-NH_2; R7574, MDS Analytical Technologies, Ontario, Canada), and 3 μM ATP (Sigma-Aldrich), unless otherwise noted. The total reaction volumes were 20 μL, and 10 μM compounds were pre-incubated with IKK2 enzyme for 10 min before the substrate and ATP were added. For the TR-FRET reaction, 60 μL detection mixture (1:600 dilution of IMAP binding reagent and 1:400 dilution of Terbium donor supplied by MDS Analytical Technologies) were added 15 h before reading the plate. The energy transfer signal was measured in a multi-label counter using the TR-FRET option (Victor II, PerkinElmer Oy, Turku, Finland). The counter setting was 340 nm excitation, 100-μs delay, and dual-emission collection for 200 μs at 495 and 520 nm. The energy transfer signal data were used to calculate the percentage inhibition and IC_{50} values.

Cell-based Assay for Pro-Inflammatory Cytokines. Eight weeks after the primary type II collagen immunization, the mouse spleens were collected for cell preparation and washed twice with PBS. The spleens were minced and the red blood cells lysed using 0.83% ammonium chloride. The cells were filtered through a cell strainer and centrifuged at 1300 rpm at 4 °C for 5 min. The cell pellets were re-suspended in RPMI 1640 medium (Sigma Aldrich) and plated in 24-well plates (Corning, NY, USA) at a concentration of 1×10^6 cells/well. Isolated splenocytes were cultured with inhibitors for 72 h. The amount of TNFα in the culture supernatants was measured by enzyme-linked immunosorbent assay (ELISA). Antibodies directed against mouse TNFα and against biotinylated anti-mouse TNFα were used as the capture and detection antibodies, respectively. Alkaline phosphatase (Sigma Aldrich) was used for the chromogenic reaction. The amounts of TNFα present in the test samples were determined from standard curves constructed with serial dilutions of recombinant murine TNFα (R&D Systems). The absorbance at 405 nm was determined using an ELISA microplate reader (Molecular Devices, Sunnyvale, CA, USA).

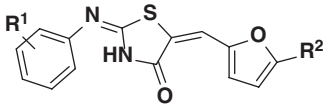
Results and Discussion

2-Phenylimino-5((5-phenylfuran-2-yl)methylene)thiazolidin-4-one derivatives (**11−23**) were synthesized and biologically evaluated for their activity against IKK2. The IKK2 assay was performed using the TR-FRET method on the IMAP platform.[10] After the percent inhibition of IKK2 was measured for each compound at a single concentration (10 μM) as a primary screening, compounds showing over 50% inhibition were evaluated for the half maximal inhibitory concentration (IC_{50}). The inhibitory activities of 2-phenyllimino-thiazolidin-4-one derivatives (**11−23**) with various phenyl ring substitutions at the 5-position within the furanylmethylene group are summarized in Tables 1−3. In Table 1, the results of the (2-phenylimino-5-phenylfuranylmethylene)thiazolidin-4-one derivatives with various substituents R^1 and R^2 (**11a−f**, **12b, 12c**, **13b**, **13f**, **13h**, **13g**, **14**, **14g, 14h**, **15g**) are summarized. The substituent R^1 including the chloro, hydroxy, carbamoyl, and cyano groups was introduced at the 2 or 3 position on the phenylimino moiety, and R^2 composed of nitrophenyl, aminophenyl, acetaminophenyl, carbamoylphenyl, and hydroxyphenyl groups was attached to the 5-position on the furanylmethylene moiety.

Based on our results, we examined the SAR of the 2-phenylimino-5((5-phenylfuran-2-yl)methylene)thiazolidin-4-one derivatives. As seen in Table 1, four compounds (**11b**, **13f**, **14h** and **14g**) showed over 60% inhibition against IKK2 at a concentration of 10 μM. Among them, **14h** (IC_{50} = 1.63 μM) was the most potent, although **11b** showed the highest percent inhibition. When R^1 is the 2-chloro substituent, the activity of compound **11a** bearing an electron-withdrawing nitro group at the 2-position decreased compared to that of **11b** having the same nitro group at the 4-position in phenyl of R^2. Compound **12b** having 3-chloro on R^1 also showed decreased activity compared to **11b**. With hydroxyl, carboxamino, and cyano groups in R^1, the inhibitory potency decreased. The combination of hydroxyl and carboxamino groups of two substituent, such as **13f** (R^1 = 3-OH, R^2 = 4-$CONH_2$Ph, IC_{50} = 5.24 μM) and **14h** (R^1 = 3-$CONH_2$, R^2 = 4-OHPh, IC_{50} = 1.63 μM), showed good potency compared to the same substituents on both sides including **13h** (R^1 = 3-OH, R^2 = 4-OH) and **14g** (R^1 = 3-$CONH_2$, R^2 = 4-$CONH_2$Ph). The 4-position substituent in R^2 has a positive effect on the inhibitory activity compared to that with the 3-position substituent (carboxamino for **13g** vs. **13f**; hydroxyl for **14** vs. **14h**). The carboxamino group in the 4-position based on benzophthalimide of the hit compound **2** improved the potency compared to the other functional groups. Particularly, the inhibitory potency decreased significantly when the aminophenyl (**11d**) or acetaminophenyl group (**11e**) was introduced at the 4-position of the R^2 substituent.

Therefore, we fixed the 4-position with a carboxaminophenyl group in R^2 and examined the effect of the methoxy group in R^1. The attachment position of the methoxy group in R^1 was varied, such as at the 2-position (**16f**) or 3-position on the phenyl ring; the carbon length was increased, such as an

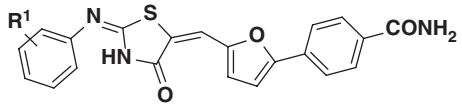
Table 1. IKK2 inhibitory activities of (2-phenylimino-5-phenylfuranylmethylene)thiazolidin-4-one derivatives.



Compound	R^1	R^2	% Inhibition (10 μM)	IC_{50} (μM)
11a	2-Cl	2-NO_2Ph	4.0	NT
11b	2-Cl	4-NO_2Ph	71.9	5.9
11c	2-Cl	4-Cl,2-NO_2Ph	32.9	12.1
11d	2-Cl	4-NH_2Ph	0	NT
11e	2-Cl	4-NHAcPh	0	NT
11f	2-Cl	4-$CONH_2$Ph	18.3	NT
12b	3-Cl	4-NO_2Ph	25.7	NT
12c	3-Cl	4-Cl,2-NO_2Ph	9.4	NT
13b	3-OH	4-NO_2Ph	12.9	NT
13h	3-OH	4-OHPh	18.3	NT
13g	3-OH	3-$CONH_2$Ph	39.3	NT
13f	3-OH	4-$CONH_2$Ph	65.7	5.24
14	3-$CONH_2$	3-OHPh	1.9	NT
14h	3-$CONH_2$	4-OHPh	66.9	1.63
14g	3-$CONH_2$	3-$CONH_2$Ph	35.1	NT
15g	3-CN	3-$CONH_2$Ph	66.3	2.51
Bayer 5a[11]	—	—	98.2	0.17
	0.17	0.17		

NT, not determined.

Table 2. IKK2 inhibitory activities of 2-phenylimino-5-[(4-carboxaminophenyl)furanylmethylene)]thiazolidin-4-one derivatives.

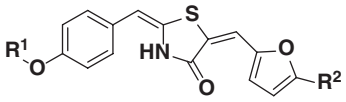


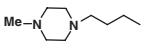
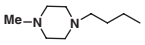
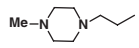
Compound	R^1	% Inhibition (10 μM)	IC_{50} (μM)
16f	2-OMe	53.8	NT
17f	3-OMe	80.8	1.72
18f	3-OEt	52.1	>10
19f	2,4-$(OMe)_2$	85.5	0.94
21e	2,3,4-$(OMe)_3$	58.6	5.04
Bayer 5a[11]	—	98.2	0.17

NT, not determined.

ethoxy group (**18f**); and more methoxy groups were attached such as a dimethoxy (**19f**) and trimethoxy group (**21e**) (Table 2). When the methoxy group (**17f**) rather than the hydroxyl group (**13f**) was introduced, the inhibition potency increased. Two methoxy groups improved the potency.

Table 3. IKK2 inhibitory activities of *N*-methylpiperazine-substituted 2-alkyloxyphenylimino-5-(4-phenylfuranylmethylene)] thiazolidin-4-one derivatives.



Compound	R^1	R^2	% Inhibition (10 μM)	IC_{50} (μM)
22c		3-ClPh	76.8	5.64
22f		4-$CONH_2$Ph	80.2	3.98
23c		3-ClPh	81.0	5.53
Bayer 5a[11]	—	—	98.2	0.17

Finally, compound **19f** having the 2,4-dimethoxy group in R^1 was the most active compound among this series (IC_{50} = 0.94 μM). This compound (**19f**), with its combination of the structural characteristics of the two virtual hit compounds (**1** and **2**), showed improved potency compared to that of each virtual hit compound individually. The ethoxy group (**16f**) or trimethoxy (**21e**) substituents resulted in a minor reduction in potency, showing 52.1 and 58.6% inhibition, respectively.

The various methylene unit-bearing *N*-methylpiperazine groups, which have positive effects on pharmacokinetic properties, were introduced at R^1 instead of the methoxy group (**22c**, **22f**, and **23c**). With the *N*-methylpiperazine group, which bears multiple methylene units, as the R^1 substituent, most compounds showed good inhibition of more than 76%, and the potency was sustained (**22f**, IC_{50} = 3.98 μM) (Table 3). Additionally, some of the selected potent compounds (**13f**, **17f**, and **22f**) were evaluated for IL-17, CCK-8, and TNF-α activity, which are NF-κB-dependent pro-inflammatory cytokines (Figure 2). The inhibition analysis of TNF-α production was performed in spleen cells from a collagen-induced arthritis (CIA) mouse model.

Compound **22f** showed comparable activity to the reference compound (**Bayer 5a**) in CCK-8 under both conditions, and IL-17 production excluded TNF-α reduction, although **13f** and **17f** did not show activity against the pro-inflammatory cytokines CCK-8, IL-17, and TNF-α.

Conclusions

2-Phenylimino-5((5-phenylfuran-2-yl)methylene)thiazolidin-4-one derivatives were prepared and evaluated for biological activity to explore their use as lead compounds and therapeutics for anti-inflammatory disease. Compound **19f** with a dimethoxy group in R^1 and 4-carboxaminophenyl in R^2 was the most potent *in vitro* IKK2 enzyme (IC_{50} = 0.94 μM), and compound **22f** with an *N*-methyl piperazine group bearing multiple methylene units in R^1 showed good activity against

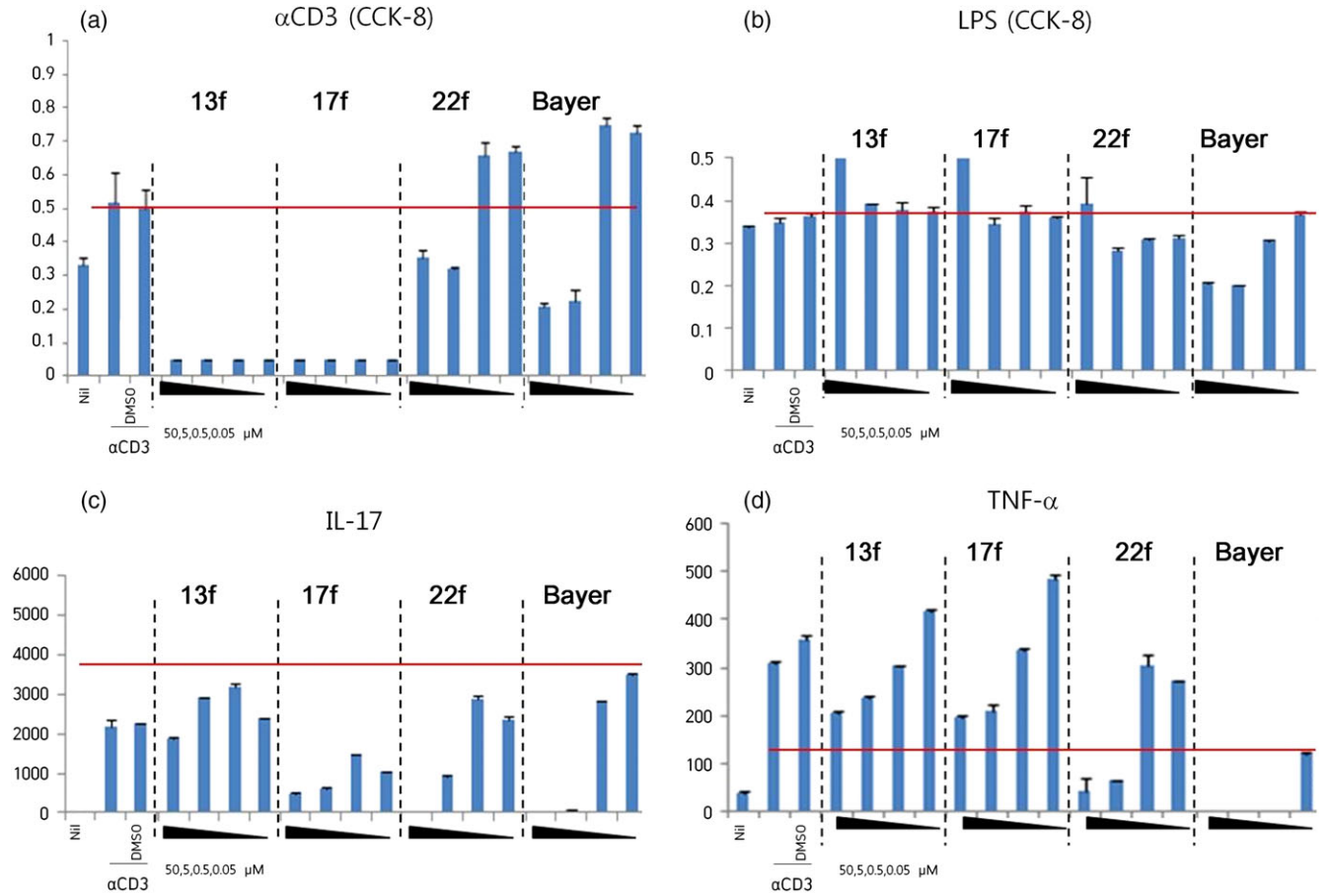


Figure 2. Activity of NF-κB pro-inflammatory cytokines including αCD3-stimulated CCK-8 (a), LPS-stimulated CCK-8 (b), IL-17 (c), and TNF-α (d).

NF-κB-dependent pro-inflammatory mediators. To identify promising leads, further optimization studies on the selectivity over other kinases such as IKK1, p38α, p38β, JNK1, JNK2, and JNK3 as well as the inhibitory activity of IKK2 are required.

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