Synthesis and Structure–Activity Relationship Study of Triazine-Based Inhibitors of the DNA Binding of NF-κB

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Nuclear transcription factor nuclear factor-kappa B (NF-κB) has diverse pathophysiological functions, and NF-κB inhibitors are considered to be candidates for multiple therapeutic applications. We previously reported a novel triazine-based NF-κB inhibitor, 2-anilino-4,6-dichloro-1,3,5-triazine (NI241), that directly inhibits DNA binding of NF-κB. Here, we report synthesis of a series of triazine derivatives and evaluation of their structure–activity relationships for NF-κB inhibition. We found that 2-amino-4,6-dichloro-1,3,5-triazine substructure is essential for the inhibitory activity of the lead compound NI241, and modification of NI241 by introduction of an m-methoxy substituent on the phenyl ring afforded the more potent derivative 28. The structure–activity relationships identified in this study suggested a possible mechanism of irreversible NF-κB inhibition by NI241, and should be helpful in the design of other NF-κB inhibitors.

Key words nuclear factor-kappa B (NF-κB); DNA binding; inhibitor; triazine

Results and Discussion

Chemistry NI241 consists of three parts: dichlorotriazine, a phenyl ring, and the linking secondary amino group. C-Chlorinated triazine is chemically reactive toward nucleophiles. Here, we focused on pyrimidine and triazine derivatives bearing dichloro groups or other functional group(s) such as alkoxy or amino groups (Fig. 1). Since our previous studies on docking simulation of NI241 to NF-κB suggested the importance of both the molecular shape of NI241 and a secondary amino group that interacts with amino acid residues of NF-κB, we also examined various N-substituents on 2-amino-4,6-dichloro-1,3,5-triazine.

Synthesis of pyrimidine derivatives is illustrated in Chart 1. Aromatic nucleophilic substitution reactions using aniline and 2,4,6-trichloropyrimidine gave anilinopyrimidines 2 and 3. Further substitution of 2 or 3 using alkoxides afforded compounds 4–8. The use of excess alkoxide afforded di-alkoxylated compounds 5 and 8. Amination of 2 or 3 using 4-methoxybenzylamine gave compounds 9 or 11, respectively. Cleavage of the 4-methoxybenzyl group of 9 and 11 under acidic conditions gave compounds 10 and 12, respectively. Methylpyrimidine derivative 14 was prepared from 4,6-dichloro-2-methylpyrimidine (13) and aniline (Chart 1).

Synthesis of 1,3,5-triazine derivatives is illustrated in Charts 2 and 3. Nucleophilic substitution reaction of 2,4,6-tri-
chloro-1,3,5-triazine (15) by aniline gave mono-substituted compound 16 (NI241) and disubstituted compound 17. Further substitution of 16 with various nucleophiles afforded mono-chlorotriazine derivatives 18–23. Compounds 24 and 25 were prepared from compound 18 by amination using methylammonium chloride or dimethylammonium chloride, respectively (Chart 2). Various 2,4-dichloro-1,3,5-triazine derivatives were synthesized in one step by substitution of 15 using corresponding amines (Chart 3).

**Biological Evaluation** The inhibitory activity of synthesized compounds toward DNA binding of NF-κB was evaluated by means of electrophoretic mobility shift assays (EMSA) using Alexa680-labeled NF-κB probe and His-tagged p50 recombinants (Tables 1, 2). All 4-phenylaminopyrimidine derivatives exhibited only weak activities, significantly lower than that of NI241 (Table 1). As for the 2-phenylaminopyrimidines, 4-ethoxy derivative 7 exhibited moderate activity, and p-methoxybenzylamino derivative 11 exhibited weak activity. However, the activity of these compounds was lower than that of NI241. These results suggested that the 1,3,5-triazine substructure is essential for the inhibitory activity of NI241. Therefore, we investigated structural development based on 1,3,5-triazine compound.

Table 3 summarizes the inhibitory activity of 2-phenylamino-1,3,5-triazine derivatives. Compounds bearing a bulky substituent, such as n-butoxy (19), phenoxy (20) or pyrrolidinyl (23), exhibited quite low activity. Though methoxy derivative 18 exhibited moderate activity, the lead compound 16 (NI241) exhibited the most potent inhibitory activity among the synthesized compounds. These results suggest that 2,4-dichloro-1,3,5-triazine substructure is the essential pharmacophore for NF-κB inhibitory activity of NI241, and aromatic nucleophilic substitution of NI241 decreased the activity.

Thus, we next investigated the inhibitory activity of various 2,4-dichloro-1,3,5-triazine derivatives toward DNA binding of NF-κB. Table 4 summarizes the results of EMSA evaluation. Most of the synthesized compounds exhibited inhibitory activity, supporting the hypothesis that the 2,4-dichloro-1,3,5-triazine substructure is the key pharmacophore for this activity. N-Phenyl derivatives including naphthyl derivative 37 and tetrahydroquinoline derivative 39 exhibited significant activity, whereas N-alkyl derivatives exhibited moderate activity. N-Methylation of secondary amines NI241 and 30, yielding 26 and 31, respectively, increased the inhibitory activity. The results suggested that aromatic and bulky amines are favorable for the inhibitory activity. Finally we examined the IC50 values of the most potent compounds. Table 5 summarized the IC50 values of the selected compounds. m-Methoxy derivative 28 exhibited the most potent inhibitory activity, showing an approximately 50% increase of potency over the lead compound NI241.

In our previous report, the binding mode of NI241 to NF-κB was simulated in silico and it was suggested that the N–H hydrogen of the aminophenyl group and two nitrogen atoms of triazine adjacent to the amino group form hydrogen bonds to NF-κB. However, the present structure–activity relationship data indicate that the N–H hydrogen is not important, and that the whole 2,4-dichloro-1,3,5-triazine substructure is required...
for inhibitory activity of N1241. Dichloro-1,3,5-triazine is an electrophilic species that could react with nucleophilic targets in proteins or water molecules in assay media. Thus, we speculate that N1241 and its derivatives function as irreversible inhibitors by reacting with NF-κB protein, or activated by conversion into hydrated form. These are also possible reasons of low biological activity of 2 and 3, the dichloropyrimidine derivatives with lower activity, corresponding to N1241. Further binding simulation studies based on the present results are in progress.

**Conclusion**

We investigated the SAR of the NF-κB DNA-binding inhibitor N1241. Its 2,4-dichloro-1,3,5-triazine substructure was identified as the essential pharmacophore for this activity. Based on the structure of N1241, we developed the more potent inhibitor 28. Since NF-κB has diverse pathophysiological functions, the SAR information obtained in this study is expected to contribute to the development of novel NF-κB inhibitors as candidates for multiple therapeutic applications.
### Table 1. Inhibitory Activity of 4-Phenylaminopyrimidine Derivatives Determined by EMSA

| Compound | R<sup>1</sup> | R<sup>2</sup> | Inhibition (%)<sup>a</sup> |
|----------|---------------|---------------|----------------------------|
| NI241    | —             | —             | 40                         |
| 2        | Cl            | Cl            | <5                         |
| 4        | MeO           | Cl            | <5                         |
| 5        | i-PrO         | Or-Pr         | 11                         |
| 9        | NH–CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>–p-OMe | Cl | 10                         |
| 10       | NH<sub>2</sub> | Cl            | 7                          |
| 14       | Me            | Cl            | 12                         |

<sup>a</sup> Inhibition ratio was determined from the decrease of Alexa680-labeled NF-κB probe at the concentration of 100 µM bound to His-tagged p50 recombinants.

### Table 2. Inhibitory Activity of 2-Phenylamino-1,3,5-triazine Derivatives Determined by EMSA

| Compound | R<sup>1</sup> | R<sup>2</sup> | Inhibition (%)<sup>a</sup> |
|----------|---------------|---------------|----------------------------|
| NI241    | —             | —             | 40                         |
| 3        | Cl            | Cl            | <5                         |
| 6        | MeO           | Cl            | <5                         |
| 7        | EtO           | Cl            | 31                         |
| 8        | i-PrO         | Or-Pr         | <5                         |
| 11       | NH–CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>–p-OMe | Cl | 22                         |
| 12       | NH<sub>2</sub> | Cl            | 14                         |

<sup>a</sup> Inhibition ratio was determined from the decrease of Alexa680-labeled NF-κB probe at the concentration of 100 µM bound to His-tagged p50 recombinants.

### Table 3. Inhibitory Activity of 2-Phenylamino-1,3,5-triazine Derivatives Determined by EMSA

| Compound | R<sup>1</sup> | R<sup>2</sup> | Inhibition (%)<sup>a</sup> |
|----------|---------------|---------------|----------------------------|
| 16 (NI241) | Cl           | Cl           | 51                         |
| 17       | NHPh          | Cl           | 15                         |
| 18       | MeO           | Cl           | 31                         |
| 19       | n-BuO         | Or-n-Bu      | 5                          |
| 20       | PhO           | Cl           | 9                          |
| 21       | MeNH          | Cl           | 14                         |
| 22       | MeN           | Cl           | 18                         |
| 23       | Pyrrolidin-1-yl | Or-Pr    | 11                         |
| 24       | MeO           | NHMe         | <5                         |
| 25       | MeO           | NMe<sub>2</sub> | 22                       |

<sup>a</sup> Inhibition ratio was determined from the decrease of Alexa680-labeled NF-κB probe at the concentration of 100 µM bound to His-tagged p50 recombinants.

### Table 4. Inhibitory Activity of 2-Amino-4,6-dichloro-1,3,5-triazine Derivatives Determined by EMSA

| Compound | R<sup>1</sup> | R<sup>2</sup> | Inhibition (%)<sup>a</sup> |
|----------|---------------|---------------|----------------------------|
| 16 (NI241) | Ph           | H            | 51                         |
| 26       | Ph            | Me           | 57                         |
| 27       | o-OMe-C<sub>6</sub>H<sub>4</sub> | H | 52                         |
| 28       | m-OMe-C<sub>6</sub>H<sub>4</sub> | H | 63                         |
| 29       | p-OMe-C<sub>6</sub>H<sub>4</sub> | H | 51                         |
| 30       | Bn            | H            | 31                         |
| 31       | Bn            | Me           | 49                         |
| 32       | Et            | Et           | <5                         |
| 33       | t-Bu          | H            | 51                         |
| 34       | n-Hex         | H            | 29                         |
| 35       | c-Hex         | H            | 7                          |
| 36       | Furfuryl      | H            | 59                         |
| 37       | 1-Naphthyl    | H            | 62                         |
| 38       | -{(CH<sub>2</sub>)<sub>3</sub>−} | H | 47                         |
| 39       | -C<sub>6</sub>H<sub>4</sub>-o-(CH<sub>2</sub>)<sub>3</sub>− | H | 55                         |

<sup>a</sup> Inhibition ratio was determined from the decrease of Alexa680-labeled NF-κB probe at the concentration of 100 µM bound to His-tagged p50 recombinants.
Experimental

General Methods All reagents were purchased from Sigma-Aldrich Chemicals Co., Tokyo Kasei Co., Wako Pure Chemical Industries, Ltd., and Kanto Chemical Co., Inc. Silica gel column chromatography was purchased from Kanto Chemical Co., Inc. 1H-NMR spectra were recorded at 400 MHz on a Bruker Avance 400 spectrometer or 500 MHz on a Bruker Avance 500 spectrometer. 13C-NMR spectra were recorded on at 125 MHz on a Brucker Avance 500 spectrometer. Chemical shifts are reported as parts per million (ppm) relative to chloroform (7.26 ppm for 1H-NMR and 77.16 ppm for 13C-NMR) or dimethylsulfoxide (DMSO) (2.50 ppm for 1H-NMR and 39.52 ppm for 13C-NMR). Data are reported as follows; chemical shift, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sex, sextet; sep, septet; br, broad; m, multiplet) coupling constants (Hz), integration. Melting points were taken on a Yanagimoto micro melting point apparatus. Mass spectral data were obtained with a JEOL AX-505 spectrometer. Elemental analyses were performed at 50°C for 2 h. The contents were poured into saturated aqueous ammonium chloride and extracted with ethyl acetate. Recrystallization of the residue gave 6 (quant.) as colorless plates (hexane); mp 105.0–105.9°C; 1H-NMR (CDCl3, 400 MHz) δ: 7.59 (dd, 2H, J = 8.7, 1.1 Hz), 7.34 (td, 2H, J = 7.1, 1.0 Hz), 7.22 (brs, 1H), 6.22 (s, 1H), 3.97 (s, 3H); 13C-NMR (CDCl3, 125 MHz) δ: 171.2, 164.5, 159.0, 138.8, 128.9, 123.1, 119.4, 97.9, 54.3. Anal. Calcd for C10H11N3O: C, 56.26; H, 4.31; N, 17.82.

4-Chloro-6-ethoxy-2-phenylaminopyrimidine (7) Sodium hydride (60% dispersion in mineral oil) (33.6 mg, 0.84 mmol) in ethyl acetate (6 mL) was added to 3 (70 mg, 0.29 mmol) at 0°C under Ar. The mixture was stirred at room temperature for 5h, then poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. Purification of the residue by silica gel flash chromatography (eluent: hexane–ethyl acetate 5:1) gave 7 (quant.) as pale-yellow needles (hexane); mp 109.1–111.2°C; 1H-NMR (CDCl3, 400 MHz) δ: 7.57 (dd, 2H, J = 8.8, 1.2 Hz), 7.33 (td, 2H, J = 7.2, 0.8 Hz), 7.06 (tt, 1H, J = 7.6, 0.8 Hz), 7.08–7.04 (brs, 1H), 6.20 (s, 1H), 4.41 (q, 2H, J = 7.21 Hz), 1.40 (t, 3H, J = 7.2 Hz); 13C-NMR (CDCl3, 125 MHz) δ: 170.9, 159.6, 158.5, 138.5, 129.0, 123.2, 119.5, 98.2, 63.4, 143. Anal. Calcd for C10H9Cl2N2O: C, 57.72; H, 4.84; N, 16.83. Found: C, 57.65; H, 4.80; N, 16.93.
in 2-propanol (3 mL) was further added in one portion. The resulting mixture was stirred at 100°C for 24 h, then poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. Purification of the residue by silica gel flash chromatography (eluent: hexane–ethyl acetate 20:1) gave 8 (56%) as a colorless oil; 1H-NMR (CDCl₃, 400 MHz) δ: 7.59 (dd, 2H, J = 8.8, 1.2 Hz), 7.30 (td, 2H, J = 8.0, 1.2 Hz), 7.00 (tt, 1H, J = 7.4, 1.2 Hz), 6.83 (brs, 1H), 5.48 (s, 1H), 5.23 (sep, 2H, J = 7.4 Hz), 1.35 (d, 12 H J = 6.4 Hz); 13C-NMR (CDCl₃, 125 MHz) δ: 171.1, 158.8, 139.8, 128.7, 118.8, 82.8, 69.0, 22.0.

4-Chloro-2-(4-methoxybenzylamino)-6-phenylaminopyrimidine (9) 4-Methoxybenzylamine (68.6 mg, 0.50 mmol) and Et₃N (63.3 mg, 0.63 mmol) were added to 2 (100 mg, 0.42 mmol) in THF (4 mL) at 0°C. The mixture was stirred at room temperature for 18 h, then poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. Purification of the residue by silica gel chromatography (eluent: hexane–ethyl acetate 8:1 to 6:1) gave 9 (53%) as colorless crystals (dichloromethane–hexane); mp 143.8–146.3°C; 1H-NMR (CDCl₃, 400 MHz) δ: 7.35 (td, 2H, J = 7.0, 2.0 Hz), 7.24 (dd, 2H, J = 6.6, 1.1 Hz), 6.57 (brs, 1H), 6.04 (s, 1H), 5.27 (s, 1H), 4.53 (dd, 2H, J = 5.8 Hz), 3.80 (s, 3H); 13C-NMR (CDCl₃, 125 MHz) δ: 162.4, 161.8, 158.8, 138.0, 130.9, 129.3, 128.7, 124.7, 114.0 93.1, 55.3, 44.9. Anal. Calcd for C₁₈H₁₇ClN₄: C, 54.43; H, 4.11; N, 25.39. Found: C, 54.29; H, 4.26; N, 25.52.

4-Chloro-2-methyl-6-phenylaminopyrimidine (14) Aniline (560 µL, 6.13 mmol) was added dropwise to 4,6-dichloro-2-methylpyrimidine (1.0 g, 6.13 mmol) in ethanol (30 mL) at 0°C. The mixture was stirred at room temperature for 19 h, then poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. Purification of the residue by silica gel chromatography (eluent: hexane–ethyl acetate 5:1) gave 14 (53%) as colorless needles (hexane); mp 109.1–111.2°C; 1H-NMR (CDCl₃, 400 MHz) δ: 7.42 (dd, 2H, J = 7.6, 0.8 Hz), 7.24 (dd, 2H, J = 8.8, 1.2 Hz), 7.24 (tt, 1H, J = 7.6, 0.8 Hz), 6.87 (brs, 1H), 6.54 (s, 1H), 2.54 (s, 3H); 13C-NMR (CDCl₃, 125 MHz) δ: 167.4, 161.3, 160.9, 136.5, 129.9, 129.6, 126.3, 123.4, 99.9, 25.2. Anal. Calcd for C₁₈H₁₄ClN₂: C, 60.14; H, 4.59; N, 19.13. Found: C, 60.36; H, 4.79; N, 19.15.

2,4-Dichloro-6-phenylamino-3,5-triazine (16) and 2-Chloro-4,6-bis(phenylamino)-1,3,5-triazine (17) Aniline (0.98 µL, 10.85 mmol) was added dropwise to cyanuric chloride (2.0 g, 10.85 mmol) in acetone (60 mL) at 0°C. Sodium methoxide (22.1 mg, 0.41 mmol) was added to 2 (50 mg, 0.21 mmol) in methanol (2.0 mL) at 0°C. The mixture was stirred at room temperature for 18 h, then poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. Purification of the residue by silica gel chromatography (eluent: hexane–ethyl acetate 8:1 to 6:1) gave 16 (53%) and 17 (10%). 16: colorless crystals (ethyl acetate–hexane); mp 134.1–140.6°C; 1H-NMR (CDCl₃, 400 MHz) δ: 7.54 (dd, 2H, J = 8.8, 1.2 Hz), 7.49 (brs, 1H), 7.42 (td, 2H, J = 7.2, 5.6 Hz), 7.23 (tt, 1H, J = 7.6, 1.2 Hz). 13C-NMR (CDCl₃, 125 MHz) δ: 171.4, 170.2, 164.1, 135.7, 129.3, 125.9, 121.2. Anal. Calcd for C₁₈H₁₂ClN₄: C, 44.84; H, 3.51; N, 23.24. Found: C, 45.13; H, 2.69; N, 23.43. 17: colorless crystals (ethyl acetate–hexane); mp 201.8–204.0°C; 1H-NMR (CDCl₃, 400 MHz) δ: 7.54 (d, 4H, J = 8.0 Hz), 7.36 (t, 4H, J = 7.6 Hz), 7.29 (brs, 2H, J = 7.2 Hz), 7.16 (t, 2H, J = 7.4 Hz). 13C-NMR (CDCl₃, 125 MHz) δ: 168.6, 164.0, 137.0, 129.1, 121.4, 121.4. Anal. Calcd for C₁₈H₁₂ClN₄: C, 60.51; H, 4.06; N, 23.52. Found: C, 60.75; H, 4.18; N, 23.65.
sodium sulfate and concentrated. Purification of the residue by silica gel chromatography (eluent: hexane–ethyl acetate 7:1) gave 18 (78%) as colorless needles (ethyl acetate–hexane); mp 174.2–176.8°C; 1H-NMR (CDCl₃, 400 MHz) δ: 7.56 (dd, 2H, J = 8.8, 1.2 Hz), 7.38 (td, 2H, J = 7.6, 2.01 Hz), 7.41–7.36 (brs, 1H), 7.17 (t, 1H, J = 7.6, 0.81 Hz), 4.04 (s, 3H); 13C-NMR (CDCl₃, 125 MHz) δ: 171.6, 170.7, 165.2, 136.7, 129.2, 124.9, 120.8, 55.8. Anal. Calcd for C₂₈H₂₈N₄O₂: C, 70.74; H, 6.24; N, 21.84. Found: C, 70.76; H, 6.30; N, 21.81.

2,4-Di-n-butoxy-6-phenylamino-1,3,5-triazine (19) Sodium hydride (60% dispersion in mineral oil, 18.0 mg, 0.45 mmol) in 1-butanol (2 mL) was added to 16 (101 mg, 0.42 mmol) at 0°C under Ar. The mixture was stirred at room temperature for 1 h and at 40°C for 1 h, then sodium hydride (60% dispersion in mineral oil, 33.4 mg, 0.84 mmol) suspended in 1-butanol (2 mL) was further added in one portion. The resulting mixture was stirred at 40°C for 4 h, then poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. Purification of the residue by silica gel chromatography (eluent: hexane–ethyl acetate 5:1) gave 22 (88%) as colorless plates (hexane); mp 243.9–245.3°C; 1H-NMR (DMSO-D₆, 373 K, 400 MHz) δ: 8.97 (br s, 1H), 7.60 (d, 1H, J = 7.6 Hz), 7.19 (t, 1H, J = 7.6 Hz), 7.08 (t, 2H, J = 7.6 Hz), 6.78 (t, 2H, J = 7.6 Hz); 13C-NMR (CDCl₃, 125 MHz) δ: 168.9, 168.1, 166.2, 164.1, 163.6, 139.4, 129.1, 123.3, 120.5, 179.2, 178.6. Anal. Calcd for C₃₀H₂₅N₅O: C, 70.74; H, 4.28; N, 29.72. Found: C, 51.17; H, 4.45; N, 29.74.

2-Chloro-4-dimethylamino-6-phenylamino-1,3,5-triazine (22) Dimethylammonium chloride (33.0 mg, 0.40 mmol) and Et,N (57 µL, 0.41 mmol) were added to 16 (100.2 mg, 0.41 mmol) in 2-propanol (2 mL) at 0°C. The mixture was stirred at room temperature for 4 h, then poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. Purification of the residue by silica gel chromatography (eluent: hexane–ethyl acetate 10:1) gave 22 (85%) as colorless needles (ethyl acetate–hexane); mp 162.2–163.4°C; 1H-NMR (CDCl₃, 400 MHz) δ: 7.57 (dd, 2H, J = 8.8, 1.2 Hz), 7.34 (td, 2H, J = 7.2, 2.01 Hz), 7.09 (t, 1H, J = 7.4, 1.2 Hz), 6.70 (brs, 1H), 3.20 (s, 6H); 13C-NMR (CDCl₃, 125 MHz) δ: 169.0, 165.2, 163.4, 138.0, 128.9, 123.7, 120.1, 36.8, 36.7. Anal. Calcd for C₁₅H₁₁ClN₄O: C, 52.91; H, 5.15; N, 27.15. Found: C, 52.85; H, 4.95; N, 27.10.

2-Chloro-4-phenylamino-6-(pyrrolidin-1-yl)-1,3,5-triazine (23) Pyrrolidine (45 µL, 0.54 mmol) was added to a solution of 16 (101 mg, 0.42 mmol) in dichloromethane (2 mL) at 0°C. The mixture was stirred at room temperature for 15 min, then poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. Purification of the residue by silica gel chromatography (eluent: hexane–ethyl acetate 7:1) gave 23 (34%) as colorless needles (ethyl acetate–hexane); mp 191.0–192.0°C; 1H-NMR (CDCl₃, 400 MHz) δ: 7.40 (td, 2H, J = 7.4, 0.91 Hz), 7.33 (dd, 2H, J = 7.2, 2.0 Hz), 7.08 (tt, 1H, J = 7.6, 1.2 Hz), 7.00 (brs, 1H), 3.63 (t, 4H, J = 6.8 Hz), 1.99 (t, 4H, J = 3.4, 3.2 Hz); 13C-NMR (CDCl₃, 125 MHz) δ: 168.7, 163.2, 1629, 139.1, 123.6, 119.8, 46.8, 46.6, 25.2, 25.0. Anal. Calcd for C₁₉H₂₀ClN₃O: C, 56.63; H, 5.12; N, 25.40. Found: C, 56.62; H, 5.17; N, 25.43.

2-Methylammonium chloride (36.1 mg, 0.42 mmol) and Et,N (70 µL, 0.50 mmol) were added to 18 (99.9 mg, 0.42 mmol) in methanol (2 mL) at 0°C. The mixture was stirred at room temperature for 2 h and at 40°C for 4 h, then methylammonium chloride (34.5 mg, 0.51 mmol) and Et,N (70 µL, 0.50 mmol) were further added. The resulting mixture was refluxed for 1 h, then poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. Purification of the residue by silica gel chromatography (eluent: hexane–ethyl acetate 5:1) gave 24 (88%) as colorless crystals (ethyl acetate–hexane); mp 163.1–164.5°C; 1H-NMR (DMSO-d₆, 373 K, 400 MHz) δ: 9.06 (brs, 1H), 7.73 (d, 2H, J = 8.0 Hz), 7.25 (t, 2H, J = 8.1 Hz), 7.01 (brs, 1H), 6.95 (t, 1H, J = 7.4 Hz), 3.83 (s, 3H), 2.84 (s, 3H, J = 4.8 Hz); 13C-NMR (CDCl₃, 125 MHz) δ: 162.1, 157.3–155.5, 139.9, 129.9, 127.3, 120.5, 36.8, 36.7. Anal. Calcd for C₁₅H₁₂ClN₄O: C, 57.13; H, 5.67; N, 30.28. Found: C, 57.31; H, 5.65; N, 30.28.

2-Dimethylammonium chloride (69.0 mg, 0.85 mmol) and Et,N (120 µL, 0.80 mmol) were added to a solution of 18 (101 mg, 0.43 mmol) in methanol (2 mL) at 0°C. The mixture was stirred at room temperature for 2 h and at 50°C for 2 h, then methylammonium chloride (38.2 mg, 0.47 mmol) and Et,N (60.0 µL, 0.43 mmol) were added in one portion. The resulting mixture was refluxed for 1 h, then poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. Purification of the residue by silica gel chromatography (eluent: hexane–ethyl acetate 5:1) gave 25 (78%) as
colorless needles (ethyl acetate–hexane); mp 163.1–164.5°C; 
^1H-NMR (CDCl$_3$, 400 MHz) δ: 7.61 (dd, 2H, J=8.8, 1.2 Hz), 7.32 (td, 2H, J=7.2, 2.0 Hz), 7.04 (tt, 1H, J=7.2, 1.2 Hz), 6.85 (brs, 1H), 3.94 (s, 3H), 3.19 (s, 6H); ^13C-NMR (CDCl$_3$, 125 MHz) δ: 170.9, 166.6, 165.0, 139.0, 128.8, 122.8, 119.8, 54.0, 36.6, 36.3. Anal. Calcd for C$_9$H$_8$N$_2$O$_2$: C, 58.76; H, 6.16; N, 28.55. Found: C, 58.90; H, 6.04; N, 28.66.

2,4-Dichloro-6-(methylphenyl)amino-1,3,5-triazine (26)
N-Methylaniline (58 µL, 0.54 mmol) and Et$_3$N (75 µL, 0.54 mmol) were added dropwise to a solution of cyanuric chloride (101 mg, 0.55 mmol) in acetone (2 mL) at 0°C under Ar. The mixture was stirred at room temperature for 2 h, and then poured into water. The precipitate was collected by filtration, washed with water, dried, and recrystallized to gave 26 (91%) as colorless prisms (CH$_2$Cl$_2$–hexane); mp 134.0–132.7°C; ^1H-NMR (CDCl$_3$, 400 MHz) δ: 7.46 (td, 2H, J=7.8, 2.0 Hz), 7.36 (tt, 1H, J=7.4, 2.0 Hz), 7.24 (dd, 2H, J=7.2, 1.2 Hz), 3.55 (s, 3H); ^13C-NMR (CDCl$_3$, 125 MHz) δ: 170.3, 170.0, 165.1, 142.0, 129.5, 127.8, 126.1, 39.2. Anal. Calcd for C$_9$H$_8$Cl$_2$N$_2$: C, 47.12; H, 3.33; N, 21.85. Found: C, 47.08; H, 3.16; N, 21.96.

**General Procedure for the Synthesis of Compounds 27–29** Anisidine (1 eq) was added dropwise to a solution of cyanuric chloride (1 eq) in acetone (2 mL) at 0°C. The mixture was stirred at room temperature for 2 h. After the completion of the reaction, the mixture was evaporated. The crude product was purified by silica gel chromatography (eluent: hexane–ethyl acetate 5:1) and then recrystallized.

2.4-Dichloro-6-(2-methoxyphenyl)amino-1,3,5-triazine (27): Yield: 86%; pale-yellow plates (ethyl acetate–hexane); mp 179.4–181.2°C; ^1H-NMR (CDCl$_3$, 400 MHz) δ: 8.31 (dd, 1H, J=8.0, 1.6 Hz), 8.20 (brs, 1H), 7.15 (td, 1H, J=7.8, 1.2 Hz), 7.04 (td, 1H, J=7.8, 1.2 Hz), 6.94 (dd, 1H, J=8.0, 1.2 Hz), 3.91 (s, 3H); ^13C-NMR (CDCl$_3$, 125 MHz) δ: 171.1, 171.0, 148.9, 125.7, 125.5, 121.2, 120.9, 110.5, 56.7. Anal. Calcd for C$_9$H$_6$Cl$_2$N$_2$O: C, 49.00; H, 3.27; N, 20.82. Found: C, 49.19; H, 3.64; N, 20.92.

2.4-Dichloro-6-diethylamino-1,3,5-triazine (28): Yield: 54%; colorless crystals (hexane); mp 78.0–78.8°C; ^1H-NMR (CDCl$_3$, 400 MHz) δ: 3.63 (q, 4H, J=7.2 Hz), 1.22 (t, 6H, J=7.2 Hz); ^13C-NMR (CDCl$_3$, 125 MHz) δ: 169.0, 163.0, 41.6, 11.7. Anal. Calcd for C$_9$H$_8$Cl$_2$N$_2$: C, 38.03; H, 4.56; N, 25.34. Found: C, 38.30; H, 4.56; N, 25.52.

2.4-Dichloro-6-(3-buty1amino)-1,3,5-triazine (29): Yield: 65%; colorless plates (hexane); mp 129.4–132.0°C; ^1H-NMR (CDCl$_3$, 400 MHz) δ: 5.78 (brs, 1H), 1.46 (s, 9H).

2.4-Dichloro-6-n-hexylamino-1,3,5-triazine (30): Yield: 56%; colorless needles (hexane); mp 54.8–55.7°C; ^1H-NMR (CDCl$_3$, 400 MHz) δ: 3.47 (q, 2H, J=6.8 Hz), 1.60 (quint, 2H, J=7.2 Hz), 1.40–1.26 (m, 6H, J=6.8 Hz).

2.4-Dichloro-6-cyclohexylamino-1,3,5-triazine (31): Yield: 40%; colorless oil; ^1H-NMR (CDCl$_3$, 400 MHz) δ: 5.76 (brs, 1H), 3.93–3.88 (m, 8H), 2.01–1.96 (m, 2H), 1.77–1.70 (m, 2H), 1.67–1.61 (m, 1H), 1.47–1.36 (m, 2H), 1.29–1.20 (m, 3H); ^13C-NMR (CDCl$_3$, 125 MHz) δ: 171.1, 170.0, 165.1, 50.3, 32.6, 25.4, 24.5. Anal. Calcd for C$_9$H$_8$Cl$_2$N$_2$: C, 47.08; H, 3.16; N, 21.96. Found: C, 47.05; H, 3.22; N, 21.99.

2.4-Dichloro-6-(piperidin-1-yl)-1,3,5-triazine (32): Yield: 40%; colorless plates (hexane); mp 88.9–89.4°C; ^1H-NMR (CDCl$_3$, 400 MHz) δ: 3.81 (t, 4H, J=5.6 Hz), 1.75–1.69 (m, 2H), 1.67–1.61 (m, 4H); ^13C-NMR (CDCl$_3$, 125 MHz) δ: 170.3, 163.7, 45.5, 25.8, 24.4. Anal. Calcd for C$_9$H$_8$Cl$_2$N$_2$: C, 41.22; H, 4.32; N, 23.98. Found: C, 41.19; H, 4.68; N, 24.04.

**General Procedure for the Synthesis of Compounds 36, 37 and 39** Amine (1 eq) in tetrahydrofuran was added dropwise to cyanuric chloride (1.2 eq) and K$_2$CO$_3$ (1 eq) in tetrahydrofuran at 0°C. The mixture was stirred at room temperature for 3–4 h then poured into water and extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate and concentrated. The crude product was purified by silica gel chromatography (eluent: hexane–ethyl acetate) and then recrystallized.
2,4-Dichloro-6-(1,2,3,4-tetrahydroquinolin-1-yl)-1,3,5-triazine (39): Yield: 93%; orange needles (hexane–ethyl acetate); mp 159.2–160.6°C; 1H-NMR (CDCl$_3$, 400 MHz) δ: 7.72 (d, 1H, J = 8.0 Hz), 7.27–7.16 (m, 3H), 4.07 (t, 2H, J = 6.4 Hz), 2.83 (t, 2H, J = 6.8 Hz), 2.05 (quint, 2H, J = 6.5 Hz); 13C-NMR (CDCl$_3$, 125 MHz) δ: 170.5, 170.4, 164.1, 136.7, 132.4, 129.0, 126.2, 126.0, 125.1, 45.7, 26.8, 23.6. Anal. Calcd for C$_{12}$H$_{10}$Cl$_2$N$_4$: C, 51.27; H, 5.59; N, 19.93. Found: C, 51.38; H, 3.60; N, 20.08.

Electrophoretic Mobility Shift Assay (EMSA)
Alexa680-labeled NF-κB probe was prepared by annealing with Alexa680-labeled single-stranded oligonucleotide and unlabeled complementary single-stranded oligonucleotide: 5'-Alexa680-AGTTGAGGGGACTTTCC
CAGGC-3' (sense) and 5'-GCCTGGGAAAGTCCC
CTCAACT-3' (antisense). The underlines show the sequence of the κB site. His-tagged NF-κB p50 recombinants were produced in E. coli and purified with HisTrap HP (GE Healthcare). Reaction mixtures containing binding buffer (15 mM Tris–HCl (pH 7.5), 75 mM NaCl, 1.5 mM ethylenediamine tetraacetic acid (EDTA), 1.5 mM dithiothreitol, 7.52% glycerol, and 0.3% Nonidet P-40), 0.5 µg of poly(dI-dC)·(dI-dC) and 62.5 ng of His-tagged p50 recombinants were left to stand on ice for 10 min, and then the candidate inhibitors were added and the mixtures were incubated at room temperature. After 30 min, 20 nM Alexa680 labeled NF-κB probe was added and incubation was continued at room temperature for 30 min. The samples in a volume of 10 µL were loaded on native 5% polyacrylamide gel prepared in 0.5×TBE and electrophoresed at 120 CV for 90 min. The gels were scanned and analyzed with an Odyssey Infrared Imaging System (LI-COR).

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