Nuclear Factor-κB Mediated Inhibition of Cytokine Production by Imidazoline Scaffolds

Daljinder K. Kahlon,[†] Theresa A. Lansdell,[†] Jason S. Fisk,[†] Christopher D. Hupp,[†] Timothy L. Friebe,[‡] Stacy Hovde,[†,§] A. Daniel Jones,[†,§] Richard D. Dyer,[∥] R. William Henry,[§] and Jetze J. Tepe*[,†]

Department of Chemistry, Michigan State University, East Lansing, Michigan 48824, Department of Biochemistry & Molecular Biology, Michigan State University, East Lansing, Michigan 48824, TCH Pharmaceuticals Inc., Ann Arbor, Michigan 48104

Received October 17, 2008

The mammalian nuclear transcription factor NF-κB is responsible for the transcription of multiple cytokines, including the pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6). Elevated levels of pro-inflammatory cytokines play an important role in the pathogenesis of inflammatory disorders such as rheumatoid arthritis (RA). Inhibition of the pro-inflammatory transcription factor NF-κB has therefore been identified as a possible therapeutic treatment for RA. We describe herein the synthesis and biological activity of a series of imidazoline-based scaffolds as potent inhibitors of NF-κB mediated gene transcription in cell culture as well as inhibitors of TNF-α and IL-6 production in interleukin 1 beta (IL-1β) stimulated human blood.

Introduction

Rheumatoid arthritis (RA[a]) is a common and often debilitating human autoimmune disease that involves chronic inflammation of joints, tissues, and organs.[1] Compared to tissues in a normal joint, RA joint tissues have increased cell densities with an abundance of neutrophils, macrophages, and T lymphocytes. Analysis of the synovial fluid from patients with RA has revealed that this tissue is rich in primarily two cytokines: tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β).[2−4] Elevated production of both TNF-α and IL-1β have been found in primary fibroblast-like synoviocytes from patients with rheumatoid arthritis (RA) as well as osteoarthritis (OA). Furthermore, cultured rheumatoid synovial cells continuously expressed IL-1 and TNF mRNA, indicating that these cytokines play important roles in the pathology of rheumatoid arthritis.[5] It is well-documented that TNF-α and IL-1β also act as pivotal signaling agents for the production of many additional cytokines, such as IL-6,[6−8] via the activation of the mammalian nuclear transcription factor, NF-κB.[9−12] IL-6 is found in abundance in RA synovium, and due to its role in B cell and hematocytic cell growth, differentiation, and proliferation of synovial fibroblasts, IL-6′s overproduction contributes to many RA symptoms.[13−15] The activation of this pro-inflammatory cytokine signaling cascade induces the release of matrix metalloproteinases (MMPs), which lead to the destruction of bone and cartilage, resulting in permanent tissue damage.[16]

TNF-α and IL-1β are considered classical (or canonical) activators of the NF-κB pathway.[17−20] NF-κB is formed from various members of the Rel family of transcription factors (NF-κB1(p50), NF-κB2(p52), c-Rel, RelA(p65), and RelB) as either a homodimer or heterodimer, where the RelA(p65)/p50 heterodimer is the most abundant.[17] Each NF-κB dimer has unique transcriptional activity, as transcriptional activators (RelA(p65)/p50, p50/c-rel, RelA(p65)/RelA(p65), and p65/c-rel) and as repressors (p50/p50 and p52/p52).[18] NF-κB is sequestered in the cytoplasm by the IκBs in normal nonstimulated cells.[21] Upon cellular stimulation, IκB is phosphorylated and subsequently polyubiquinated and proteolytically degraded by the 26S proteasome.[21−24] These events unmask the nuclear localization signal (NLS) of NF-κB, allowing it to translocate into the nucleus.[23] Following its nuclear translocation, NF-κB binds to DNA and induces gene transcription of a wide variety of genes, including those responsible for proinflammatory responses. The levels of NF-κB-DNA binding are consequently much greater in tissues from RA patients compared to control patients.[20,25,26] Hence, regulation of the NF-κB mediated expression of cytokines has been actively pursued as a means of therapeutic intervention in these types of inflammatory disorders.[27,28]

Pharmacologic intervention in RA was improved drastically with the advent of biologicals that target TNF-α, providing a proof-of-principle for therapeutic intervention in RA by targeting TNF-α.[29−31] Unfortunately, these and alternative treatment options for RA are limited and suffer from high costs, undesirable methods of administration, and lack of data on long-term safety, tolerability, and sustained efficacy. In addition, variability in responses to these anti-inflammatory drugs is found due to the complex network of alternative cytokine-mediated pathways.[32] Inhibition of pro-inflammatory transcription factors, such as NF-κB, may therefore represent a better alternative to modulate the complex cytokine network that induces inflammatory responses.[33] Consequently, inhibition of NF-κB by a small molecule would represent an attractive therapeutic alternative to the current clinical options.[12,34]

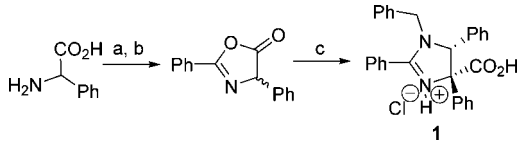
We previously described the synthesis and biological activity of a series of imidazolines as potent small molecule inhibitors of NF-κB transcription, with imidazoline **1** being the most effective in cell culture.[35,36] The imidazolines are formed via trimethylsilyl chloride mediated 1,3-dipolar cycloaddition of oxazol-5-(4*H*)-ones and imines (Scheme 1).[37,38] Even though the parent molecule **1** displayed excellent activity in cell culture, additional studies found that the imidazoline scaffold **1** was fairly unstable upon standing and easily converted to a zwitterionic form **2** (Scheme 2, X-ray crystal structures of **1** and **2**

* To whom correspondence should be addressed: Phone: +1-517-355-9715. Fax: +1-517-353-1793. E-mail: tepe@chemistry.msu.edu.
[†] Department of Chemistry, Michigan State University.
[‡] Current address: Chemistry Department, Eastern Michigan University.
[§] Department of Biochemistry & Molecular Biology, Michigan State University.
[∥] TCH Pharmaceuticals Inc.

[a] Abbreviations: RA, rheumatoid arthritis; TNF-α, tumor necrosis factor α; IL-1β, interleukin-1β; OA, osteoarthritis, IL-6, interleukin-6; NLS, nuclear localization signal; TFAA, trifluoroacetic anhydride; TMS, trimethylsilyl; EDCI, 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide; DMAP, 4-dimethylaminopyridine.

10.1021/jm8013162 CCC: $40.75
Published on Web 02/16/2009

Scheme 1. Synthesis of the *trans*-Imidazolines[a]



[a] (a) Benzoyl chloride, 1 N NaOH (aq), overnight; (b) TFAA, CH_2Cl_2, 2 h, room temperature, 77% overall; (c) TMSCl, imine, CH_2Cl_2, reflux, overnight, 68%.

are provided in the Supporting Information). Zwitterion **2** is readily decarboxylated to form the ylide **3**,[39] which provided a mixture of three products: the *cis*-dihydroimidazoline **4**, the *trans*-dihydroimidazoline **5**, and the fully aromatized imidazole **6** (Scheme 2). Furthermore, the *cis*- and *trans*-dihydroimidazolines (**4** and **5**) isomerized upon standing and dehydrogenated to the imidazole **6** at room temperature when exposed to polar solvents such as DMSO. This *cis*/*trans* isomerization and oxidation of the decarboxylated form of diaryl-substituted imidazolines is well-documented,[40−42] and thus this zwitterion induced decarboxylation needed to be addressed to pursue its further clinical potential. The instability of imidazoline **1** results in undesirable pharmacological properties and could be the reason for the inconsistent activity seen when imidazoline **1** was tested in cell culture and whole blood (Table 1). Fortunately, chemical manipulation and derivatization of the carboxyl moiety readily restores stability and potency. In this paper, we describe the synthesis of derivatives of imidazoline **1** aimed to prevent the aforementioned degradation and the new compounds were evaluated for their stability and inhibition of NF-κB mediated TNF-α and IL-6 production. Biological evaluation indicated that three analogues substantially inhibited NF-κB mediated transcription in cell culture and inhibited TNF-α and IL-6 production in IL-1β stimulated human blood at low micromolar concentrations.

Synthesis and Biological Evaluation

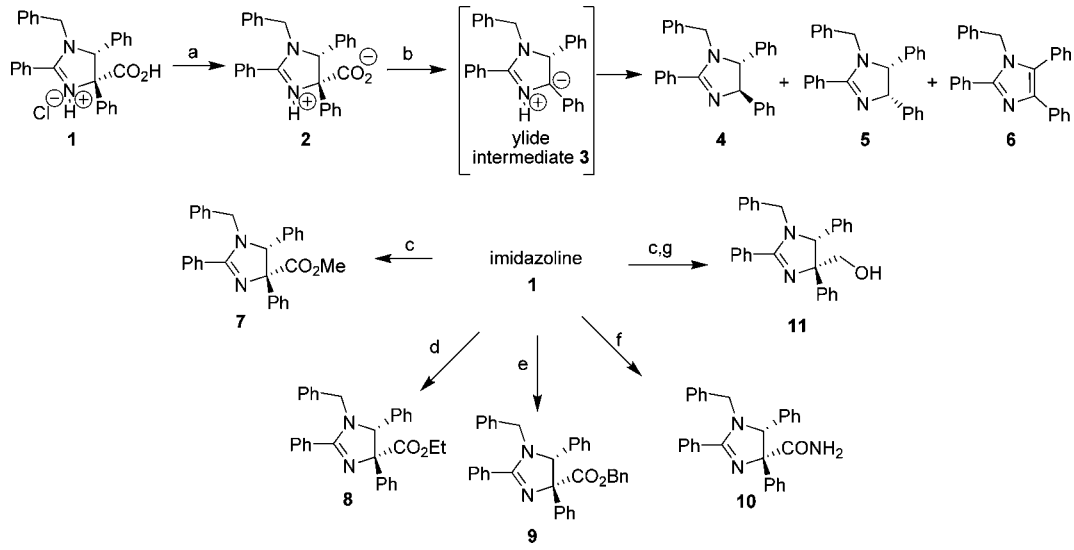
Synthesis of Imidazoline Derivatives. We envisioned that the replacement of the carboxylic acid in **1** with esters or a less acidic alcohol or amide functionalities would avoid the formation of the zwitterion. The imidazoline scaffold was prepared via our previously reported protocol using a silicon mediated 1,3-dipolar cycloaddition (Scheme 1).[37,38] The resulting imidazoline·HCl salt **1** was treated with $TMSCHN_2$ to yield the methyl ester **7** in excellent yield (Scheme 2). The ethyl ester **8** was prepared via the acid chloride. To form the benzyl ester **9**, the imidazoline **1** was coupled with benzyl alcohol, EDCI, and DMAP. To determine the importance of the ester moiety, imidazoline **1** was converted to the amide **10** using similar coupling conditions and $(NH_4)_2CO_3$. Finally, the ester was reduced to the alcohol **11** with lithium aluminum hydride to study the significance of the carbonyl moiety for activity (Scheme 2).

Inhibition of NF-κB-Mediated Gene Transcription. The ability of the imidazolines **1**, **2** and **4**−**11** to modulate NF-κB-mediated gene transcription was evaluated using a luciferase reporter assay in human cervical epithelial (HeLa) cells with a stably transfected NF-κB-luc gene. These cells maintain, through hygromycin selection, a chromosomal integration of a luciferase reporter construct regulated by multiple copies of the NF-κB response element. The cells were activated with TNF-α (25 ng/mL) in the presence or absence of imidazolines **1**, **2**, **4**−**11**. Because activation of the NF-κB pathway requires proteasomal degradation of IκB, a classic proteasome inhibitor **12** (*N*-CBz-Leu-Leu-leucinal, also known as MG-132, Figure 1B)[43] was used as positive control and DMSO (vehicle) served as the negative control. Luciferase production was evaluated after 8 h, and all samples were normalized to the TNF-α activation control. Treatment of HeLa/NF-κB-luc cells with imidazolines without any TNF-α activation did not induce any significant amount of luciferase activity at 20 μM, indicating that the imidazolines alone did not stimulate the NF-κB pathway. Pretreatment of the cells with the imidazolines followed by TNF-α stimulation resulted in a dose-dependent decrease in luciferase production for several of the analogues of **1**, **7**−**9**, whereas the zwitterion **2** was devoid of activity (Table 1). While the decarboxylated products **4** and **5** displayed significantly reduced activity, the imidazoline ester **8** was the most active alternative to compound **1** with an EC_{50} value in HeLa cells of 2.5 μM (Figure 1A). The imidazole **6**, amide **10**, and alcohol **11** were significantly less active, with EC_{50} values at or greater than 20 μM. To verify that the results for luciferase production in this integrated cell line were due to inhibition of NF-κB and not to cell death, HeLa cells were transiently transfected with 6× κB driven reporter gene pNF-κB-luc (Stratagene) and the internal control plasmid pRL-TK (Stratagene), which provides low levels of *Renilla* luciferase expression. After activation with TNF-α in the presence or absence of imidazoline **8**, luciferase production was assayed and the data was normalized to the *Renilla* luminescence (see Supporting Information). The two luciferase assays corresponded well with one another, confirming the results from the luciferase assay using the stable HeLa/NF-κB-luc cell line. These results indicate that the ester moiety represented a comparable alternative to imidazoline **1** for NF-κB inhibition.

Inhibition of TNF-α and IL-6 Production by Imidazolines in Human Whole Blood. The results from cell culture prompted the analysis of these imidazolines for their anti-inflammatory potential in whole blood. The imidazolines were subsequently evaluated for their ability to inhibit a NF-κB mediated cytokine response in IL-1β stimulated human blood. Human blood samples were pretreated with imidazolines **4**−**11** for 2 h followed by IL-1β stimulation. Plasma was harvested 22 h after stimulation and IL-1β induced TNF-α production was measured using a human TNF-α ELISA (R&D Systems) assay. The circulating TNF-α levels in IL-1β stimulated blood samples were significantly higher than in unstimulated or the vehicle treated blood. Pretreatment of the blood for 2 h with the imidazolines **4**−**11**, followed by IL-1β stimulation, resulted in a strong dose-dependent inhibition of TNF-α production, as compared to the vehicle treated control (Figure 1C). The amide **10** and the alcohol **11** were inactive (Table 1). Therefore, imidazolines **7**−**9** were effective in decreasing TNF-α levels, presumably via inhibition of NF-κB mediated gene transcription.

To evaluate the effect of inhibiting TNF-α production on other downstream pro-inflammatory cytokines, such as IL-6, the same plasma samples were subjected to an IL-6 ELISA (R&D Systems). As seen with the TNF-α response, IL-1β stimulation caused a robust increase in IL-6 production when compared to the unstimulated control or the vehicle-only treated blood samples. Pretreatment of the blood for 2 h with the imidazolines **4**, **5**, **7**−**11**, followed by IL-1β stimulation, resulted in a strong dose-dependent inhibition of IL-6 production, as compared to the vehicle treated control (Figure 1D). Consistent with the TNF-α data as well as the NF-κB mediated luciferase activity, compounds **10** and **11** were significantly less active. The reduction of IL-6 levels clearly indicates that inhibition of NF-κB activation causes a decline in the production of additional pro-inflammatory cytokines. Even though the decarboxylated

Scheme 2. Synthesis of Imidazolines Derivatives[a]



[a] (a) $NaHCO_3$ (aq). (b) DMSO, rt or THF, reflux. (c) $TMSCHN_2$, benzene:MeOH (9:1), 0 °C. (d) (1) $(COCl)_2$, CH_2Cl_2, 0 °C; (2) EtOH; (e) EDCI, DMAP, benzyl alcohol, CH_2Cl_2. (f) EDCI, HOBt, DIPEA, $(NH_4)_2CO_3$, THF. (g) $LiAlH_4$, THF.

Table 1

compd	HeLa NF-κB-luc EC_{50}[a], μM	whole blood TNF-α IC_{50}[b], μM	whole blood IL-6 IC_{50}[c], μM
1	0.95[d]	>20	>20
4	4.6	NT[e]	2.5
5	11.0	NT[e]	2.4
6	>20	NT[e]	NT[e]
7	7.5	2.8	3.0
8	2.5	1.2	0.8
8a (*R*,*R*)	1.6	0.6	0.2
8b (*S*,*S*)	2.9	0.7	0.6
9	3.5	3.2	5.9
10	~20	>20	>20
11	>20	11.9	~20

[a] EC_{50} values for inhibition of luciferase production in pNF-κB-luc HeLa cells following TNF-α activation. [b] IC_{50} values for inhibition of TNF-α production in human whole blood following IL-1β stimulation. [c] IC_{50} values for inhibition of IL-6 production in human whole blood following IL-1β stimulation. EC_{50} and IC_{50} values were calculated from logarithmic curves (see Supporting Information for complete list of log values and standard errors). [d] Reference 35. [e] NT: Not tested.

compounds (**4** and **5**) inhibited IL-6 production in the low micromolar range, their ability to isomerize and aromatize to the imidazole **6** made them unattractive as potential drug candidates.

From this data, compound **8** was determined to be the superior compound of this series based on the activities in all three assays. Considering the difference between the types of assays, the activity of the compounds in cell culture corresponded very well with the activity in IL-1β stimulated whole blood for both TNF-α and IL-6 inhibition (Table 1). This data indicates that imidazoline **8** is a very effective inhibitor of NF-κB mediated gene transcription, resulting in a decrease in the levels of the pro-inflammatory cytokines TNF-α and IL-6 in IL-1β stimulated human blood. To demonstrate the pharmacological relevance of these imidazolines, human blood samples were first stimulated with IL-1β and then treated with imidazoline **8** at various doses (see Supporting Information). The IC_{50} value for post-treatment was comparable to that for pretreatment, suggesting the imidazolines inhibit inflammatory response after it has been initiated.

Cytotoxicity of Imidazoline 8. To exclude the possibility of decreased cytokine production due to an increase of imidazoline mediated cell death, both the HeLa cells and white blood cells were evaluated for cell death at various concentrations of imidazoline **8**. An LDH release assay showed that incubation of HeLa/NF-κB-luc cells with up to 10 μM imidazoline **8** for 8 h did not induce any significant amount of cell death (see Supporting Information). FACS analysis of these samples confirmed the cells were healthy (see Supporting Information). To confirm the cell viability data in the whole blood experiments, human white blood cell counts also indicated no significant cell cytotoxicity at 10 μM or less imidazoline **8** (see Supporting Information). Therefore, the inhibition of luciferase production and inhibition of TNF-α and IL-6 production by imidazoline **8** was not due to a decrease in cell number but rather due to inhibition of NF-κB transcription.

Stability of Imidazoline 8. Sterically accessible carboxylic esters are often susceptible to hydrolysis by blood and cellular esterases, a conversion that could present a potential problem due to the reoccurrence of the zwitterionic form **2** and hence lead to instability of the imidazoline scaffold. The ethyl ester derivative (compound **8**) was therefore incubated in whole human blood for 24 h and evaluated for hydrolysis. Compound **8** was found to be stable to blood esterolytic activity for up to 24 h at 37 °C as determined by LC/MS analysis of compound-spiked human blood. LC/MS analysis indicated no detection of cleavage products over this 24 h incubation period (see Supporting Information). These results indicated that imidazoline **8** is the active compound in the assays and not a pro-drug for imidazoline **1**.

Evaluation of Enantiomers. To optimize the anti-inflammatory efficacy of our scaffold, each enantiomer (**8a**-(*R*,*R*) and **8b**-(*S*,*S*)) of the racemic compound **8** was evaluated for its biological activity. Previously, we found that both enantiomers of compound **1** were active in cell culture, however the (*R*,*R*) enantiomer of imidazoline **1** was approximately 5-fold more potent than the (*S*,*S*) enantiomer.[36] To evaluate the two enantiomers of compound **8**, the enantiomers were readily resolved by chiral HPLC (see Supporting Information). This procedure gives excellent resolution (~5 min between enantiomers), and both enantiomers were isolated and evaluated for their activities. Similar to the imidazoline **1**, both enantiomers showed strong inhibition of NF-κB mediated gene transcription,

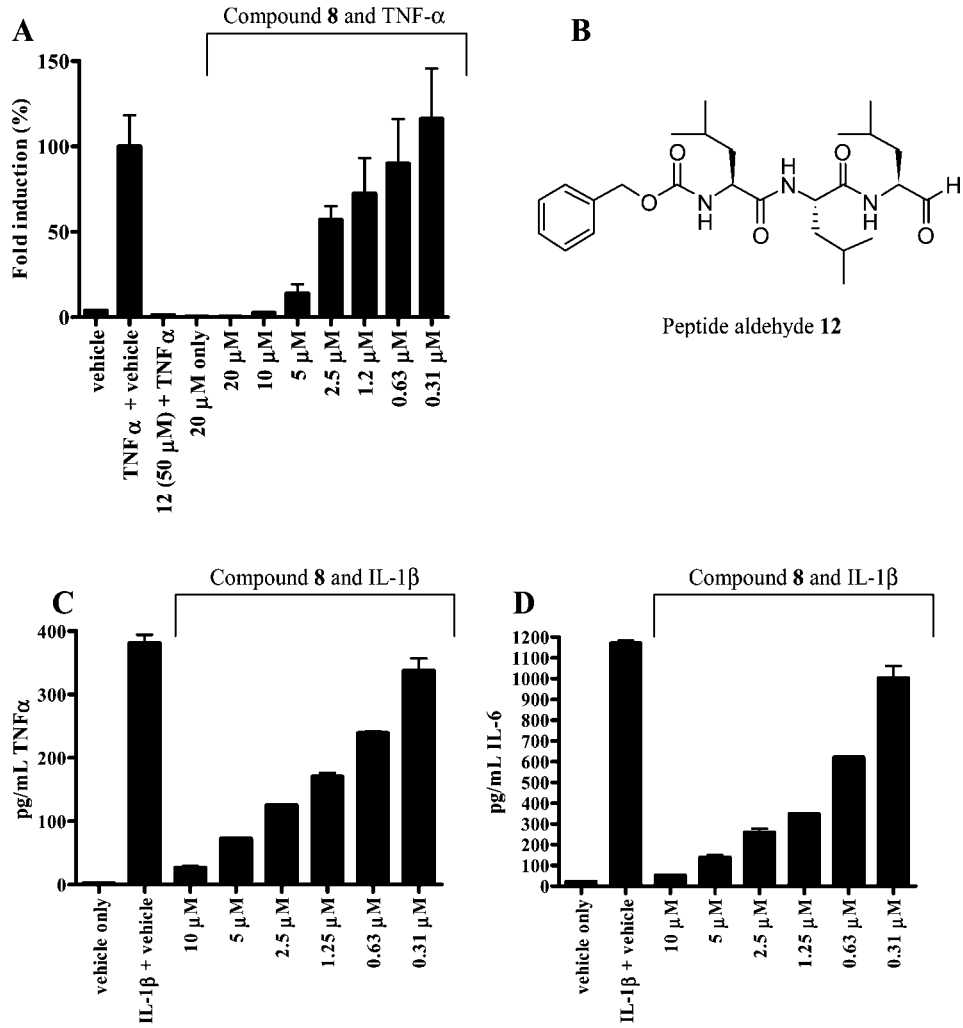


Figure 1. Dose−response activity of imidazoline **8** in (A) inhibition of luciferase production in HeLa-NF-κB-luc cells, (C) inhibition of TNF-α production in IL-1β stimulated human blood, and (D) inhibition of IL-6 production in IL-1β stimulated human blood. (A) Cells were unstimulated/stimulated with 25 ng/mL TNF-α in the absence or presence of imidazoline **8** in 1% DMSO. Fold-induction (%) of the luciferase activity, normalized for all samples to TNF-α stimulation, is shown. (B) Structure of peptide aldehyde **12**. (C) Human blood (1:10 RPMI) were unstimulated/stimulated with 200 U/mL IL-1β in the absence or presence of imidazoline **8** in 1% DMSO. Fold-induction of the TNF-α levels of stimulated (calculated from unstimulated) cells was determined. (D) Human blood (1:10 RPMI) were unstimulated/stimulated with 200 U/mL IL-1β in the absence or presence of imidazoline **8** in 1% DMSO. Fold-induction of the IL-6 levels of stimulated (calculated from unstimulated) cells was determined. All experiments were run in duplicate.

with the (*R*,*R*) enantiomer **8a** ($EC_{50} = 1.6\ \mu M$) somewhat more potent than the (*S*,*S*) enantiomer **8b** ($EC_{50} = 2.9\ \mu M$) (Table 1). The enantiomers were also tested for their ability to inhibit cytokine production in whole human blood. Once more, the (*R*,*R*) enantiomer **8a** was found to be slightly more potent than the (*S*,*S*) enantiomer **8b**. The IC_{50} values for TNF-α and IL-6 inhibition for the (*R*,*R*) enantiomer **8a** were 0.6 and 0.2 μM, respectively. The IC_{50} values for the (*S*,*S*) enantiomer were in the same range, albeit slightly less potent. These results indicate that both enantiomers of **8** are able to inhibit cytokine production at the low micromolar level, with the (*R*,*R*) enantiomer to be slightly superior.

Conclusion

The NF-κB inhibitor imidazoline **1** was previously found to be a potent inhibitor of NF-κB mediated gene transcription in cell culture. The carboxylic acid functionality of this class of compounds was found to be responsible for subsequent decomposition (Scheme 1). Herein, we described the synthesis and biological activity of structurally modified imidazolines, incapable of forming a zwitterionic species. The ethyl ester derivative **8** was found to be a potent inhibitor of NF-κB mediated gene transcription in HeLa cells with low micromolar EC_{50} values and stable against degradation and hydrolysis by esterases. The cellular activity of the imidazolines translated well in whole human blood assays, resulting in nanomolar IC_{50} values for TNF-α and IL-6 production after IL-1β mediated NF-κB stimulation. Furthermore, evaluation of isolated enantiomers revealed the (*R*,*R*) enantiomer of imidazoline **8** to be slightly more potent than the (*S*,*S*) enantiomer at inhibiting NF-κB mediated gene transcription and cytokine production. The further clinical potential of these stable noncytotoxic small molecule NF-κB inhibitors as potential anti-inflammatory agents is currently under investigation in our laboratories.

Experimental Section

General Information. All commercial reagents were purchased from commercial suppliers and used without further purification. All solvents were reagent grade. Peptide aldehyde **12** was obtained from Calbiochem (San Diego, CA), TNF-α was purchased from Invitrogen (Carlsbad, CA), and IL-1β was obtained from Roche Applied Sciences (Indianapolis, IN). THF was freshly distilled from

sodium/benzophenone under nitrogen. CH_2Cl_2 was dispensed from a delivery system, which passes the solvents through a column packed with dry neutral alumina. Melting points were obtained using an electrothermal capillary melting point apparatus and are uncorrected. Column chromatography was carried out on silica gel 60 (230−400 mesh) supplied by EM Science. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. Infrared spectra were recorded on a Nicolet IR/42 spectrometer. Proton and carbon NMR spectra were recorded on a Varian Unity Plus-500 spectrometer. High-resolution 70 eV EI mass spectra and FAB mass spectra were obtained at the RTSF Mass Spectrometry Facility of the Michigan State University, using a JEOL AX-505H and a JEOL HX-110 double focusing mass spectrometer (JEOL USA, Peabody, MA), respectively. Combustion analysis was preformed on a PerkinElmer 2400 Series II CHNS/O analyzer. All final compounds possess purity >95% as determined by combustion analysis, high-resolution mass spectrometry, or HPLC.

DL-(4*R*,5*R*)-1-Benzyl-2,4,5-triphenyl-4,5-dihydro-1*H*-imidazole-4-carboxylic Acid Hydrochloride (1). Melting point 152−155 °C. ^{1}H NMR (500 MHz, DMSO-*d*) δ 4.18 (d, 1H, J = 16.0 Hz), 4.86 (d, 1H, J = 16.0 Hz), 5.59 (s, 1H), 6.71 (d, 2H, J = 7.5 Hz), 7.10 (t, 2H, J = 7.7 Hz), 7.19 (t, 2H, J = 7.5 Hz), 7.45−7.59 (m, 7H), 7.72−7.77 (m, 4H), 7.84 (t, 1H, J = 7.2 Hz), 7.97 (d, 2H, J = 7.5 Hz), 12.86 (br s, 1H), 13.99 (br s, 1H). ^{13}C NMR (125 MHz, DMSO-*d*) δ 48.7, 73.8, 75.9, 121.4, 127.2, 128.3, 128.7, 128.9, 129.0, 129.1, 129.4, 129.5, 129.9, 132.8, 133.2, 134.3, 139.2, 165.7, 167.6.

DL-(4*R*,5*R*)-1-Benzyl-2,4,5-triphenyl-4,5-dihydro-1*H*-imidazol-3-ium-4-carboxylate (2). DL-(4*R*,5*R*)-1-benzyl-2,4,5-triphenyl-4,5-dihydro-1*H*-imidazole-4-carboxylic acid hydrochloride (**1**) was dissolved in dichloromethane. Then it was washed 2× saturated $NaHCO_3$ solution. The organic layer was separated, dried over $MgSO_4$, and concentrated in vacuo to yield the zwitterion **2** as an off-white solid; mp 119−121 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.78 (d, 1H, J = 16.0 Hz), 4.60 (d, 1H, J = 16.0 Hz), 4.91 (s, 1H), 6.60 (d, 2H, J = 7.5 Hz), 7.06 (t, 2H, J = 7.5 Hz), 7.15 (t, 1H, J = 7.5 Hz), 7.26−7.33 (m, 5H), 7.38−7.41 (m, 5H), 7.49 (t, 1H, J = 7.5 Hz), 7.54 (d, 2H, J = 7.5 Hz), 7.82 (d, 2H, J = 7.0 Hz). ^{13}C NMR (125 MHz, DMSO-*d*) δ 48.7, 75.8, 79.3, 123.3, 125.9, 127.0, 127.7, 128.2, 128.5 (2 peaks), 129.1, 129.2, 129.3, 129.3, 129.6, 133.2, 134.0, 136.2, 143.3, 165.1, 168.4.

DL-(4*R*,5*R*)-1-Benzyl-2,4,5-triphenyl-4,5-dihydro-1*H*-imidazole (4). The synthesis of compound **4** has been previously reported.[44] The title compound was isolated in small amounts via a decarboxylation of imidazoline **1**. A solution of imidazoline **1** (0.7 g, 1.62 mmol) in 100 mL of THF was refluxed for 5 h, resulting in complete decomposition of starting material. The solution was then concentrated in vacuo, resulting in a crude mixture of imidazolines **4** and **5** and imidazole **6** in a 2:1:1 ratio, respectively. The crude residue was then purified via flash column chromatography using silica gel (1:9 methanol:dichloromethane as eluant), isolating the product as a white solid (112 mg, 18%-unoptimized); mp 78−80 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.99 (d, 1H, J = 16 Hz), 4.41 (d, 1H, J = 8.5 Hz), 4.77 (d, 1H, J = 15.5 Hz), 5.07 (d, 1H, J = 8.5 Hz), 7.00−7.01 (m, 2H), 7.18−7.23 (m, 2H), 7.24−7.30 (m, 4H), 7.31−7.33 (m, 2H), 7.34−7.39 (m, 3H), 7.42−7.45 (m, 2H), 7.53−7.55 (m, 3H), 7.88−7.90 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 49.6, 72.6, 77.9, 126.7, 127.0, 127.1, 127.4, 127.7, 127.9, 128.3, 128.4, 128.6, 128.6, 128.8, 130.1, 131.3, 136.4, 141.8, 143.9, 165.9. IR (neat): 3063 cm^{-1}, 3030 cm^{-1}, 1595 cm^{-1}. HRMS (FAB): m/z calculated for $[C_{28}H_{24}N_2 + H]^+$, 389.2018; found, 389.2020.

Because of the difficulty in separation and to verify the structures of compounds **5** and **6**, each compound was prepared individually via an alternative route.

DL-(4*R*,5*S*)-1-Benzyl-2,4,5-triphenyl-4,5-dihydro-1*H*-imidazole (5). A solution of DL-(4*R*,5*S*)-2,4,5-triphenyl-4,5-dihydro-1*H*-imidazole[45] (0.3 g, 1.01 mmol) and benzyl bromide (0.2 g, 1.06 mmol) in 20 mL of anhydrous benzene was treated with triethyl amine (0.2 g, 2.02 mmol). The solution was refluxed for 15 h and then washed with saturated $NaHCO_3$ solution (2 × 20 mL) and brine solution (1 × 20 mL). The solution was then dried over Na_2SO_4 and concentrated in vacuo. The resulting crude residue was purified via flash column chromatography using silica gel (1:9 methanol:dichloromethane as eluant), affording the product as a yellow oil (77 mg, 20%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.85 (d, 1H, J = 15.5 Hz), 4.77 (d, 1H, J = 16 Hz), 4.92 (d, 1H, J = 11.5 Hz), 5.55 (d, 1H, J = 11 Hz), 6.90−6.96 (m, 5H), 6.97−7.00 (m, 4H), 7.02−7.09 (m, 3H), 7.25−7.29 (m, 3H), 7.50−7.53 (m, 3H), 7.81−7.83 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 49.0, 68.4, 72.9, 126.2, 127.1, 127.3, 127.5, 127.8, 127.9, 127.9, 128.1, 128.5, 128.6, 128.7, 130.2, 131.2, 136.6, 136.8, 139.3, 167.1. IR (neat): 3030 cm^{-1}, 2924 cm^{-1}, 1595 cm^{-1}. HRMS (FAB): m/z calculated for $[C_{28}H_{24}N_2 + H]^+$, 389.2018; found, 389.2017.

1-Benzyl-2,4,5-triphenyl-1*H*-imidazole (6). To a flame-dried 100 mL round-bottom flask was added 2,4,5-triphenylimidazole (2 g, 6.76 mmol) and anhydrous THF (60 mL). Sodium hydride (432 mg, 10.81 mmol) was then added and the mixture stirred for 5 min. Then benzyl chloride (1.24 mL, 10.81 mmol) was added dropwise and the mixture refluxed overnight under a nitrogen atmosphere. The solid precipitate was filtered off and the THF was concentrated down. The crude residue was put into solution using ethyl acetate (50 mL) and was washed with brine solution (1 × 30 mL). The organics were dried using anhydrous sodium sulfate and concentrated. The crude residue was purified by flash column chromatography using silica gel (8:2 hexane:ethyl acetate as eluant) affording the product as a white powder (1.33 g, 51%); mp 159−161 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.1 (s, 2H), 6.78−6.81 (m, 2H), 7.1−7.23 (m, 8H), 7.28−7.4 (m, 6H), 7.57−7.6 (m, 2H), 7.64−7.67 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 48.2, 125.9, 126.2, 126.7, 127.2, 128.0, 128.51, 128.52, 128.55, 128.72, 128.81, 129.0, 130.0, 130.9, 131.0, 134.4, 137.5, 138.0, 148.0. IR (NaCl) 3065 cm^{-1}, 3032 cm^{-1}, 1956 cm^{-1}, 1889 cm^{-1}, 1812 cm^{-1}, 1762 cm^{-1}. HRMS (ESI): m/z calculated for $[C_{28}H_{22}N_2 + H]^+$, 387.1861; found, 387.1873.

DL-(4*R*,5*R*)-Methyl 1-Benzyl-4,5-dihydro-2,4,5-triphenyl-1*H*-imidazole-4-carboxylate (7). A flame-dried flask under nitrogen was charged with DL-(4*R*,5*S*)-1-benzyl-4,5-dihydro-2,4,5-triphenyl-1*H*-imidazole-4-carboxylic acid (**1**) (0.50 g, 1.07 mmol) in 9:1 benzene:methanol (50 mL) and cooled to 0 °C. After 5 min, (trimethylsilyl)diazomethane (1.1 mL, 2.13 mmol) was added dropwise and then the reaction mixture was allowed to stir at 0 °C for 3 h. The reaction mixture was concentrated to minimal residue and purified by running through a small silica plug to give the product as a cream-colored solid (0.24 g, 50%); mp 114−116 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.18 (s, 3H), 3.84 (d, 1H, J = 16.0 Hz), 4.63 (d, 1H, J = 16.0 Hz), 4.91 (s, 1H), 6.73 (d, 2H, J = 7.5 Hz), 7.05 (t, 2H, J = 7.5 Hz), 7.10 (t, 1H, J = 7.5 Hz), 7.25−7.28 (m, 1H), 7.31−7.34 (m, 3H), 7.37 (d, 4H, J = 4.5 Hz), 7.47 (d, 1H, J = 1.5 Hz), 7.48(d, 1H, J = 2.0 Hz), 7.73 (m, 2H), 7.77 (d, 1H, J = 3.5 Hz), 7.78 (d, 1H, J = 1.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 48.7, 51.8, 73.9, 83.1, 126.8, 127.2, 127.4, 127.5, 128.0, 128.1, 128.3, 128.4, 128.5, 128.6, 128.9, 130.4, 130.5, 136.6, 137.9, 144.0, 165.6, 171.4. IR (NaCl): 3061 cm^{-1}, 3031 cm^{-1}, 2948 cm^{-1}, 1734 cm^{-1}, 1595 cm^{-1}. HRMS (ESI): m/z calculated for $[C_{30}H_{26}N_2O_2 + H]^+$, 447.2070; found, 447.2070.

DL-(4*R*,5*R*)-Ethyl 1-Benzyl-4,5-dihydro-2,4,5-triphenyl-1*H*-imidazole-4-carboxylate (8). Into a flame-dried flask under nitrogen was placed DL-(4*R*,5*S*)-(1-benzyl-4,5-dihydro-2,4,5-triphenyl-1*H*-imidazole-4-carboxylic acid (**1**) (20.0 g, 46.3 mmol) and dichloromethane (300 mL). After cooling the reaction mixture to 0 °C, oxalyl chloride (11.7 mL, 138.8 mmol) was added over 5 min, followed by DMF (10 μL/1 mL of dichloromethane). The reaction mixture was allowed to stir at 0 °C for 2 h, after which the solvent was removed on the rotary evaporator. The residue was placed on a vacuum line for 1 h. The flask was then placed under nitrogen and cooled to 0 °C and ethanol (400 mL) was added. After stirring for an addition 2.5 h, the ethanol was removed on the rotary evaporator and dichloromethane (300 mL) was added. The organic solution was washed with saturated $NaHCO_3$ solution (1 × 100 mL) and H_2O (1 × 100 mL), dried over $MgSO_4$, filtered, and the

solvent was removed under reduced pressure to give a yellow oil. The product was purified using flash column chromatography on silica gel (1:1 hexane:ethyl acetate as eluant) to give a white solid (16.1 g, 76%); mp 86−88 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.83 (t, 3H, J = 7.2 Hz), 3.63 (qq, 1H, J = 7.2 Hz), 3.73 (qq, 1H, J = 7.2 Hz), 3.86 (d, 1H, J = 16.0 Hz), 4.64 (d, 1H, J = 15.5 Hz), 4.96 (s, 1H), 6.78 (d, 2H, J = 7.0 Hz), 7.09 (t, 2H, J = 7.2 Hz), 7.14 (t, 1H, J = 7.2 Hz), 7.28−7.32 (m, 1H), 7.34−7.38 (m, 3H), 7.39−7.45 (m, 4H), 7.49−7.51 (m, 3H), 7.79−7.81(m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 13.4, 48.6, 60.9, 73.7, 82.8, 126.7, 127.1, 127.2, 127.3, 127.9, 128.1, 128.2, 128.3, 128.4, 128.5, 128.8, 130.2, 130.6, 136.6, 137.9, 144.1, 165.3, 170.7. IR (NaCl): 3061 cm^{-1}, 3031 cm^{-1}, 2981 cm^{-1}, 1732 cm^{-1}, 1597 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $[C_{31}H_{28}N_2O_2 + H]^+$, 461.2229; found, 461.2225.

DL-(4*R*,5*R*)-Benzyl 1-Benzyl-4,5-dihydro-2,4,5-triphenyl-1*H*-imidazole-4-carboxylate (9). To a stirred solution of DL-(4*R*,5*S*)-1-benzyl-4,5-dihydro-2,4,5-triphenyl-1*H*-imidazole-4-carboxylic acid (**1**) (200 mg, 0.43 mmol) in dichloromethane was added 1-ethyl-(3-dimethylaminopropyl) carbodiimide (120 mg, 0.64 mmol). After 5 min was added 4-(*N*,*N*-dimethylamino)pyridine (52 mg, 0.43 mmol). After stirring for an addition 10 min, benzyl alcohol (92 mg, 0.85 mmol) was added. The reaction mixture was stirred at room temperature overnight. The reaction mixture was washed with 10% HCl solution (1 × 5 mL), saturated $NaHCO_3$ solution (1 × 5 mL), H_2O (1 × 5 mL), and brine solution (1 × 5 mL). The product was extracted using dichloromethane, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The product was purified using column chromatography on silica gel (9:1 dichloromethane:ethyl acetate as eluant) to give a light-yellow oil (120 mg, 54%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.79 (d, 1H, J = 15.5 Hz), 4.50 (d, 1H, J = 12.5 Hz), 4.61 (d, 1H, J = 15.5 Hz), 4.76 (d, 1H, J = 12.5 Hz), 4.92 (s, 1H), 6.74 (d, 2H, J = 7.5 Hz), 6.95 (dd, 2H, J = 7.5, 2.5 Hz), 7.04−7.08 (m, 2H), 7.10−7.13 (m, 1H), 7.17−7.21 (m, 3H), 7.27−7.29 (m, 4H), 7.30−7.34 (m, 4H), 7.47−7.49 (m, 3H), 7.74−7.77 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 48.6, 66.6, 73.7, 82.7, 126.8, 127.1, 127.4, 127.5, 127.7, 127.9.0, 128.0 (2 peaks), 128.4 (2 peaks), 128.5, 128.6, 128.8, 130.4, 135.4, 136.3, 137.3, 143.5, 165.6, 170.3. IR (NaCl): 3063 cm^{-1}, 3032 cm^{-1}, 2924 cm^{-1}, 1740 cm^{-1}, 1593 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $[C_{36}H_{30}N_2O_2 + H]^+$, 523.2386; found, 523.2390.

DL-(4*R*,5*R*)-1-Benzyl-4,5-dihydro-2,4,5-triphenyl-1*H*-imidazole-4-carboxamide (10). To a stirred solution of DL-(4*R*,5*S*)-1-benzyl-4,5-dihydro-2,4,5-triphenyl-1*H*-imidazole-4-carboxylic acid (**1**) (100 mg, 0.21 mmol,), *N*-hydroxybenzotriazole (32 mg, 0.23 mmol), and 1-ethyl-(3-dimethylaminopropyl) carbodiimide (45 mg, 0.23 mmol) in anhydrous THF (2 mL) was added *N*,*N*-diisopropylethylamine (30 mg, 0.23 mmol). The reaction mixture was stirred at ambient temperature for 10 min. Then ammonium carbonate (50 mg, 0.64 mmol) was added in one portion and the resulting suspension was stirred at ambient temperature overnight. The reaction mixture was concentrated to minimal residue. The residue was treated with 1:1 saturated $NaHCO_3$ solution:H_2O (2 mL) and stirring was continued for 2 h. The mixture was placed into a separatory funnel, and the product was extracted using ethyl acetate, dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product was purified using column chromatography on silica gel (3:7 hexane: ethyl acetate as eluant) to give a light-yellow solid (65 mg, 71%); mp 65−68 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.80 (d, 1H, J = 16.5 Hz), 4.56 (d, 1H, J = 16.5 Hz), 4.86 (s, 1H), 5.15 (br s, 1H), 6.71 (d, 2H, J = 7.5 Hz), 7.05 (t, 2H, J = 7.5 Hz), 7.11 (t, 1H, J = 7.2 Hz), 7.26−7.28 (m, 1H), 7.30−7.36 (m, 7H), 7.50−7.54 (m, 3H), 7.71−7.73 (m, 2H), 7.79 (d, 2H, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 48.4, 74.3, 81.6, 126.3, 126.9, 127.3, 127.4, 128.1, 128.3, 128.4, 128.5, 128.8, 130.6, 136.7, 137.8, 144.8, 165.4, 173.4. IR (NaCl): 3442 cm^{-1}, 3063 cm^{-1}, 3031 cm^{-1}, 2926 cm^{-1}, 1688 cm^{-1}, 1591 cm^{-1}. HRMS (FAB): *m*/*z* calculated for $[C_{29}H_{25}N_3O + H]^+$, 432.2076; found 432.2073.

DL-(4*R*,5*R*)-1-Benzyl-4,5-dihydro-2,4,5-triphenyl-1*H*-imidazol-4-yl)methanol (11). In a flame-dried flask a suspension of lithium aluminum hydride (7 mg, 0.18 mmol) in anhydrous THF (8 mL) was cooled to 0 °C. Then a solution of DL-(4*R*,5*S*)-methyl 1-benzyl-4,5-dihydro-2,4,5-triphenyl-1*H*-imidazole-4-carboxylate (**7**) (50 mg, 0.11 mmol) in THF (3 mL) was added and the reaction mixture was stirred under nitrogen and warmed to room temperature. The reaction mixture was quenched using saturated NH_4Cl solution (3 mL). The mixture was allowed to stir for 10 min and then placed in a separatory funnel. The product was extracted using ethyl acetate (20 mL). The aqueous layer was washed with fresh ethyl acetate (5 mL). The combined organic layers were washed with brine solution, dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. The crude material was purified via column chromatography on silica gel (100% ethyl acetate as eluant) to yield a white solid (29 mg, 62%); mp 138−141 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.78 (br s, 1H), 3.47−3.50 (m, 1H), 3.53−3.56 (m, 1H), 3.87 (d, 1H, J = 15.5 Hz), 4.59 (s, 1H), 4.72 (d, 1H, J = 15.5 Hz), 6.72 (d, 2H, J = 6.5 Hz), 7.05−7.10 (m, 3H), 7.21−7.22 (m, 1H), 7.32 (t, 2H, J = 7.5 Hz), 7.35−7.41 (m, 6H), 7.54 (t, 3H, J = 3.5 Hz), 7.84−7.85 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 49.0, 67.0, 73.8, 76.9, 125.8, 126.8, 127.5, 127.6, 128.1, 128.4, 128.4, 128.5, 128.8, 128.8, 130.5, 130.7, 135.7, 135.9, 146.3, 166.5. IR (NaCl): 3183 cm^{-1} (broad), 3061 cm^{-1}, 3029 cm^{-1}, 2924 cm^{-1}, 1597 cm^{-1}. HRMS (ESI): *m*/*z* calculated for $[C_{29}H_{26}N_2O + H]^+$, 419.2123; found 419.2125.

Biological Methods. Cell Culture. The human cell line HeLa-NF-κB-luc was purchased from Panomics Inc. (Fremont, CA). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 4.5 g/L glucose, 3.7 g/L bicarbonate, and supplemented with 5% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate, 0.2 mM L-glutamine, and 100 μg/mL of hygromycin B. The human cell line HeLa was purchased from ATCC. The cells were maintained in DMEM media containing 4.5 g/L glucose, 3.7 g/L bicarbonate, and supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 mg/mL streptomycin, 1 mM sodium pyruvate, and 0.2 μM L-glutamine. Cells were cultured at 37 °C, 5% CO_2 atmosphere, 97% relative humidity and were routinely passaged by trypsin-EDTA treatment to maintain a cell density between 2 × 10^5 to 5 × 10^5.

Viability Assays. Human white blood cells were counted manually using a BD Unopette reservoir and a hemacytometer.

NF-κB-luc Reporter Assay. HeLa NF-κB-luc cells (5.0 × 10^5 cells/mL) were seeded into a 96-well white opaque plate using DMEM medium supplemented with 5% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, 1 mM sodium pyruvate, 0.2 mM L-glutamine, and 100 μg /mL hygromycin B. After 24 h, the cell culture medium was replaced with DMEM medium supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin. Cell cultures were pretreated with vehicle (1% DMSO), 50 μM peptide aldehyde **12** (positive control), or imidazoline (final concentrations were 20, 10, 5, 1, 0.5, 0.1, 0.05 μM) for 30 min at 37 °C in 5% CO_2. TNF-α was added to a final concentration of 25 ng/mL, and the samples were further incubated for 8 h at 37 °C in 5% CO_2. Cells were assayed for firefly luciferase production using the Steady-Glo luciferase reporter assay (Promega, Madison, WI) according to manufacturer's protocol. The luminescence of each well was measured using a Veritas microplate luminometer. All reported data are the average of two independent experiments unless otherwise indicated. The data was analyzed using GraphPad Prism 4.0. The data was normalized to TNF-α activation, and the EC_{50} values were calculated using the equation for the sigmodial curve for variable slope.

NF-κB-luc/Renilla-luc Dual Reporter Assay. HeLa cells (3.5 × 10^5 cells/mL) were seeded into a 6-well plate using using DMEM media supplemented with 10% fetal bovine serum and no antibiotics. Transient transfections were performed using lipofectamine 2000. Briefly, 0.8 μg of plasmid DNA was combined with 1.5 μL lipofectamine 2000. HeLa cells were transiently cotransfection with internal control pRL-TK. A constitutively activated plasmid pFC-MEKK served as positive control and pCIS-CK was the negative control. The mixture was incubated at room temperature for 20 min and mixed with cells in Opti-MEM I medium. Cells were transfected for 5−6 h at 37 °C in 5% CO_2. Cells were allowed to

grow in complete growth medium for 24 h in 5% CO_2. Cell cultures were pretreated with vehicle (1% DMSO), 50 μM peptide aldehyde **12** (positive control), or imidazoline **8** (final concentrations were 20, 10, 5, 2.5, 1.25, 0.625, 0.312 μM) for 30 min at 37 °C in 5% CO_2. TNF-α was added to a final concentration of 25 ng/mL, and the samples were further incubated for 8 h at 37 °C in 5% CO_2. Cells were assayed for both firefly luciferase production as well as *Renilla* luciferase (coreporter) using the Dual-Glo reporter assay according to the manufacturer's protocol. The results were read on Veritas microplate luminometer as relative light units. Values for the luciferase activity were normalized to the *Renilla* luciferase and analyzed using GraphPad Prism 4.0 (see Supporting Information).

Human Whole Blood IL-1β Challenge. After obtaining the appropriate approval for deidentified human cell lines, human whole blood was obtained through the Jasper Research Clinic, Kalamazoo, MI, from a single healthy, fasted human volunteer and was collected in glass citrated endotoxin-free tubes by venipuncture. Only samples with a white blood count falling within the normal range (4800−10800 white blood cells per liter) were used. To support the viability of white blood cells, blood was diluted 1:10 in RPMI-1640 media supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. Aliquots of diluted blood (1 mL) were preincubated with vehicle (0.1% DMSO, final concentration) or imidazoline (final concentrations were 10, 3, 1, 0.3, and 0.1 μM) for 2 h at 37 °C, 5% CO_2. IL-1β was added to a final concentration of 200 U/mL, and the samples were further incubated for 18 h at 37 °C, 5% CO_2. At the end of the incubation period, the blood samples were centrifuged at 3000 RPM for 5 min. The plasma was removed, snap frozen, and stored at −80 °C. TNF-α and IL-6 levels were determined by ELISA (R&D Systems, Inc.).

CytoTox-ONE Homogeneous Membrane Integrity Assay. HeLa NF-κB-luc cells (3.8×10^5 cells/mL) were seeded into a 96-well black opaque plate using DMEM media supplemented with 2% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 100 μg/mL hygromycin B. Cells were treated with either vehicle (1% DMSO) or imidazoline **8** (final concentrations were 40, 20, 10, 5, 2, 0.5, 0.2 μM) for 8 h at 37 °C, 5% CO_2. Media-only wells served as negative control to determine background fluorescence. Untreated cells with 1% DMSO served as vehicle control. The plate and CytoTox-ONE assay reagent (Promega, Madison, WI) were equilibrated to room temperature. To determine the maximum LDH release, 2 μL of lysis solution (9% w/v Triton X-100 in water) was added to high control wells. Background LDH release from tissue culture cells was considered as low (media) control. An equal volume of CytoTox-ONE assay reagent was subsequently added to each well. The contents of the plate were gently mixed for 30 s and allowed to incubate at room temperature for 10 min. The fluorescence of each well was recorded on SpectraMax M5^e with an excitation wavelength of 560 nm and an emission wavelength of 590 nm. All reported data are the average of four independent experiments unless otherwise indicated. The data was normalized to maximum LDH release (see Supporting Information).

FACS Analysis. Cells were treated with either vehicle (1% DMSO) or imidazoline **8** (final concentrations were 40, 20, 10, 5, 2, 0.5, 0.2 μM) for 8 h at 37 °C, 5% CO_2. For the analysis of cell cycle parameters, the adherent cell cultures were trypsinized and resuspended in 1 mL of 1× PBS with 50% FBS. Then the cells were fixed with 70% (v/v) ice-cold ethanol overnight. After centrifugation of the cells, the pellet was washed two times with 5 mL of ice cold 1× PBS containing 10% FBS. Then the cell pellet was resuspended in staining solution (PBS containing 50 μg propidium iodide, 10 μL 0.1 M EDTA, 14 units RNaseA). The DNA content was analyzed by flow cytometry using a FACS Vantage SE analyzer with ModFit LT software (see Supporting Information).

LC/MS Assay for Stability of Compound 8 in Whole Blood. Plasma aliquots (50 μL) from blood incubated with imidazoline **7** for different incubation times up to 24 h were added to 200 μL of ice-cold HPLC-grade acetonitrile in a 1.5 mL microcentrifuge tube. After centrifugation (5000*g*, 15 min; 4 °C), 100 μL of each supernatant was transferred to an autosampler vial containing 100 μL of 0.15% aqueous formic acid. These solutions were analyzed by LC/MS on a Waters LCT Premier mass spectrometer equipped with Shimadzu SIL-5000 autosampler and LC-20AD pumps. Analyses were performed using a Waters Atlantis dC18 column (2.1 mm × 50 mm, 3 μm particles) using a flow rate of 0.3 mL/min. The gradient consisted of 10 mM aqueous ammonium acetate (solvent A) and acetonitrile (solvent B), starting at 90% A with a linear gradient to 100% B at 20 min. Volumes of 10 μL were injected and analyzed using positive mode electrospray ionization. Extracted ion chromatograms were generated for the protonated compound **8** (*m*/*z* 461.2) and the hydrolyzed product (compound **1**, *m*/*z* 433.2). Retention times and response factors were obtained through analysis of authentic standards of compounds **1** and **8**.

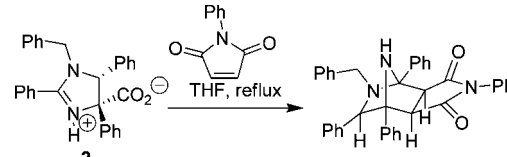
Acknowledgment. We gratefully acknowledge funds from the American Cancer Society (RSG CDD-106972), Michigan Universities Commercialization Initiative (MUCI), Michigan State University, and funding from the TCH Pharmaceuticals Inc. We also thank Rui H. Huang of Michigan State University for helpful discussions and X-ray crystallography, the Mass Spectrometry Facility at Michigan State University for assistance in acquiring HRMS data, and Dr. Louis King for assistance in acquiring FACS data.

Supporting Information Available: Experimental data for reporter assays, ELISA IL-6, ELISA TNF-α, FACS, and cell lysis experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Feldmann, M.; Brennan, F. M.; Maini, R. N. Rheumatoid Arthritis. *Cell* **1996**, *85*, 307–310.
(2) Saxne, T.; Palladino, M., Jr.; Heinegård, D.; Talal, N.; Wollheim, F. A. Detection of tumor necrosis factor α but not tumor necrosis factor β in rheumatoid arthritis synovial fluid and serum. *Arthritis Rheum.* **1988**, *31*, 1041–1045.
(3) Hopkins, S. J.; Humphreys, M.; Jayson, M. I. V. Cytokines in synovial fluid: I. The presence of biologically active and immunoreactive IL-1. *Clin. Experimental Immunol.* **1988**, *72*, 422–427.
(4) Hopkins, S. J.; Meager, A. Cytokines in synovial fluid: II. The presence of tumour necrosis factor and interferon. *Clin. Exp. Immunol.* **1988**, *73*, 88–92.
(5) Buchan, G.; Barrett, K.; Turner, M.; Chantry, D.; Maini, R. N.; Feldmann, M. Interleukin-1 and tumour necrosis factor mRNA expression in rheumatoid arthritis: prolonged production of IL-1α. *Clin. Exp. Immunol.* **1988**, *73*, 449–455.
(6) Feldmann, M.; Brennan, F. M.; Maini, R. N. Role of cytokines in rheumatoid arthritis. *Annu. Rev. Immunol.* **1996**, *14*, 397–440.
(7) Choy, E. H. S.; Panayi, G. S. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N. Engl. J. Med.* **2001**, *344*, 907–916.
(8) Firestein, G. S.; Alvaro-Garcia, J. M.; Maki, R. Quantitative analysis of cytokine gene expression in rheumatoid arthritis. *J. Immunol.* **1990**, *144*, 3347–3353.
(9) Makarov, S. S. NF-κB in rheumatoid arthritis: a pivotal regulator of inflammation, hyperplasia, and tissue destruction. *Arthritis Res.* **2001**, *3*, 200–206.
(10) Miagkov, A. V.; Kovalenko, D. V.; Brown, C. E.; Didsbury, J. R.; Cogswell, J. P.; Stimpson, S. A.; Baldwin, A. S.; Makarov, S. S. NF-κB activation provides the potential link between inflammation and hyperplasia in the arthritic joint. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 13859–13864.
(11) Jimi, E.; Aoki, K.; Saito, H.; D'Acquisto, F.; May, M. J.; Nakamura, I.; Sudo, T.; Kojima, T.; Okamoto, F.; Fukushima, H.; Okabe, K.; Ohya, K.; Ghosh, S. Selective inhibition of NF-κB blocks osteoclastogenesis and prevents inflammatory bone destruction in vivo. *Nat. Med.* **2004**, *10*, 617–624.
(12) Barnes, P. J.; Karin, M. Nuclear factor-κB—a pivotal transcription factor in chronic inflammatory diseases. *N. Engl. J. Med.* **1997**, *336*, 1066–1071.
(13) Houssiau, F. A.; Devogelaer, J.-P.; Damme, J. V.; de Deuxchaisnes, C. N.; Snick, J. V. Interleukin-6 in synovial fluid and serum of patients with rheumatoid arthritis and other inflammatory arthritides. *Arthritis Rheum.* **1988**, *31*, 784–788.

(14) Cronstein, B. N. Interleukin-6 A key mediator of systemic and local symptoms in rheumatoid arthritis. *Bulle. NYU Hosp. Joint Dis.* **2007**, *65*, S11−S15.
(15) Snick, J. V. Interleukin-6: an overview. *Annu. Rev. Immunol.* **1990**, *8*, 253–278.
(16) Chu, C. Q.; Field, M.; Allard, S.; Abney, E.; Feldmann, M.; Maini, R. N. Detection of cytokines at the cartilage/pannus junction in patients with rheumatoid arthritis: implication for the role of cytokines in cartilage destruction and repair. *Br. J. Rheumatol.* **1992**, *31*, 653–661.
(17) Siebenlist, U.; Franzoso, G.; Brown, K. Structure, regulation and function of NF-κB. *Annu. Rev. Cell Biol.* **1994**, *10*, 405–455.
(18) Ghosh, S.; May, M. J.; Kopp, E. B. NF-κB and REL proteins: evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* **1998**, *16*, 225–260.
(19) Baeuerle, P. A.; Henkel, T. Function and activation of NF-κB in the immune system. *Annu. Rev. Immunol.* **1994**, *12*, 141–179.
(20) Albert, S.; Baldwin, J. The NF-κB and IκB proteins: new discoveries and insights. *Annu. Rev. Immunol.* **1996**, *14*, 649–681.
(21) Karin, M.; Ben-Neriah, Y. Phosphorylation meets ubiqutination: the Control of NF-κB activity. *Annu. Rev. Immunol.* **2000**, *18*, 621–663.
(22) Karin, M. The beginning of the end: IκB kinase (IKK) and NFκB activation. *J. Biol. Chem.* **1999**, *274*, 27339–27342.
(23) Baeuerle, P. A.; Baltimore, D. IκB: a specific inhibitor of the NF-κB transcription factor. *Science* **1988**, *242*, 540–546.
(24) Beg, A. A.; Albert, S.; Baldwin, J. The IκB proteins: mutlifunctional regulators of Rel/NF-κB transcription factors. *Genes Dev.* **1993**, *7*, 2064–2070.
(25) Aupperle, K.; Bennett, B.; Han, Z.; Boyle, D.; Manning, A.; Firestein, G. NF-kappa B regulation by I kappa B kinase-2 in rheumatoid arthritis synoviocytes. *J. Immunol.* **2001**, *166*, 2705–2711.
(26) Han, Z.; Boyle, D. L.; Manning, A. M.; Firestein, G. S. AP-1 and NF-kappaB regulation in rheumatoid arthritis and murine collagen-induced arthritis. *Autoimmunity* **1998**, *28*, 197–208.
(27) Renard, P.; Raes, M. The proinflammatory transcription factor NFκB: a potential target for novel therapeutical strategies. *Cell Biol. Toxicol.* **1999**, *15*, 341–344.
(28) D'Acquisto, F.; May, M. J.; Ghosh, S. Inhibition of Nuclear Factor Kappa B (NF-κB): An emerging theme in anti-Inflammatory therapies. *Mol. Interventions* **2002**, *2*, 22–35.
(29) Maini, R. N.; Elliott, M. J.; Brennan, F. M.; Feldmann, M. Beneficial effects of tumour necrosis factor-alpha (TNF-α) blockade in rheumatoid arthritis (RA). *Clin. Exp. Immunol.* **1995**, *101*, 207–212.
(30) Kremer, J. M. Rational use of new and existing disease-modifying agents in rheumatoid arthritis. *Ann. Intern. Med.* **2001**, *134*, 695–706.
(31) Feldmann, M.; Maini, R. N. Anti-TNFα therapy of rheumatoid arthritis: what have we learned? *Annu. Rev. Immunol.* **2001**, *19*, 163–196.
(32) Handel, M. L.; Nguyen, L. Q.; Lehmann, T. P. Inhibition of transcription factors by anti-inflammatory and anti-rheumatic drugs: can variability in response be overcome? *Clin. Exp. Pharmacol. Physiol.* **2000**, *27*, 139–44.
(33) Tak, P. P.; Firestein, G. S. NF-kappaB: a key role in inflammatory diseases. *J. Clin. Invest.* **2001**, *107*, 7–11.
(34) Karin, M. Inflammation-Activated Protein Kinases as Targets for Drug Development. *Proc. Am. Thorac. Soc.* **2005**, *2*, 386–390.
(35) Sharma, V.; Lansdell, T. A.; Peddibhotla, S.; Tepe, J. J. Sensitization of tumor cells toward chemotheraphy: enhancing the efficacy of camptothecin with imidazolines. *Chem. Biol.* **2004**, *11*, 1689–1699.
(36) Sharma, V.; Peddibhotla, S.; Tepe, J. J. Sensitization of cancer cells to DNA damaging agents by imidazolines. *J. Am. Chem. Soc.* **2006**, *128*, 9137–9143.
(37) Peddibhotla, S.; Jayakumar, S.; Tepe, J. J. Highly diastereoselective multicomponent synthesis of unsymmetrical imidazoline scaffolds. *Org. Lett.* **2002**, *4*, 3533–3535.
(38) Peddibhotla, S.; Tepe, J. J. Multicomponent synthesis of highly substituted imidazolines via a silicon mediated 1,3-dipolar cycloaddition. *Synthesis* **2003**, *9*, 1433–1440.
(39) The proposed ylide **3** (Scheme 2) was trapped with the dipolarphile *N*-phenylmaleimide. Compound **2** was treated with *N*-phenylmaleimide in THF and refluxed for 2 h. The crude material was purified by flash column chromatography using silica gel with 2:8 ethyl acetate:hexane to 3.5:6.5 ethyl acetate:hexane as eluant. The exobicyclic product **13** was isolated in 24% yield (unoptimized conditions).



(40) Anastassiadou, M.; Baziard-Mouysset, G.; Payard, M. New one-step synthesis of 2-aryl-1*H*-imidazoles: Dehydrogenation of 2-aryl-Δ2-imidazolines with dimethylsulfoxide. *Synthesis* **2000**, *13*, 1814–1816.
(41) Matsuura, T.; Ito, Y. Photoinduced reactions. LXI. Photochemical *cis*,*trans*-isomerization of 2,4,5-triphenylimidazoles. *Chem. Lett.* **1972**, 431–434.
(42) Matsuura, T.; Ito, Y. Photochemical *cis*−*trans* isomerization of 2,4,5-triphenylimidazolines. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 3669–3674.
(43) Li, Y.-P.; Schwartz, R. J.; Waddell, I. D.; Holloway, B. R.; Reid, M. B. Skeletal muscle myocytes undergo protein loss and reactive oxygen-mediated NF-κB activation in response to tumor necrosis factor α. *J. Fed. Am. Soc. Exp. Biol.* **1998**, *12*, 871–880.
(44) Clemens, N.; Sereda, O.; Wilhelm, R. Unexpected behaviour of tosylated and acetylated imidazolinium salts. *Org. Biomol. Chem.* **2006**, *4*, 2285–2290.
(45) Braddock, D. C.; Redmond, J. M.; Hermitage, S. A.; White, A. J. P. A Convenient Preparation of Enantiomerically Pure (+)-(1*R*,2*R*)− and (−)-(1*S*,2*S*)-1,2-Diamino-1,2-diphenylethanes. *Adv. Synth. Catal.* **2006**, *348*, 911–916.

JM8013162