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Activation of NFκB is inhibited by curcumin and related enones

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Abstract—The transcription factor NFkappaB (NFκB) is up-regulated in many cancer cells where it contributes to development of the pro-survival, anti-apoptotic state. The natural product curcumin is a known inhibitor of activation of NFκB. Enone analogues of curcumin were compared with curcumin for their abilities to inhibit the TNFα-induced activation of NFκB, using the Panomics' NFκB Reporter Stable Cell Line. The enones tested included curcumin analogues that retained the 7-carbon spacer between the aromatic rings, analogues with a 5-carbon spacer, and analogues with a 3-carbon spacer. Inhibitors of NFκB activation were identified in all three series, a number of which were more active than curcumin. Enone analogues in the series with the 5-carbon spacer were especially active, including members that contained heterocyclic rings. 1,5-Bis(3-pyridyl)-1,4-pentadien-3-one was the most active analogue, $IC_{50} = 3.4 \pm 0.2$ μM. The most active analogues retain the enone functionality, although some analogues devoid of the enone functionality exhibited activity. The activity of the analogues as inhibitors of the activation of NFκB did not correlate with their anti-oxidant activity. The data suggest that the abilities of curcumin and analogues to prevent the stress-induced activation of NFκB result from the inhibition of specific targets rather than from activity as anti-oxidants.

1. Introduction

The transcription factor NFκB is an established regulator of numerous genes important in the inflammatory response. More recently however, activation of NFκB has been shown to have a role in many aspects of oncogenesis including control of apoptosis as well as regulation of cell cycling and cell migration.[1,2] Activated NFκB has been observed in many cancers and is especially important in metastasis.[3] There are five members of the NFκB family, distinguished by the presence of an N-terminal Rel homology domain: Rel (c-Rel), RelA (p65), RelB, NFκB1 (p50 and its precursor p105), and NFκB2 (p52 and its precursor p100). NFκB transcription factors are homo- or heterodimers of these members, with the p65/p50 heterodimer being the most common. NFκB members are retained in the cytosol as complexes with a set of inhibitory proteins, designated IκB. These inhibitory proteins mask a nuclear localization signal. In the classical pathway for activation of NFκB, the upstream IκB kinase complex (IKK) is first activated in response to many different signals, resulting in phosphorylation of IκB, followed by its ubiquitination and proteosomal degradation. This is followed by nuclear translocation of NFκB with resulting activation of a battery of genes, including anti-apoptotic pro-survival genes. There are also alternative pathways for activation of NFκB.[4,5]

The evidence that links activation of NFκB to oncogenesis is compelling. NFκB is activated by a number of viral transforming proteins,[6] and inhibition of NFκB activation through expression of a dominant negative IKK can block cell transformation.[7] NFκB activation protects cells from apoptosis induced by cancer chemo-therapeutics and oncogenes,[8] and activation of NFκB promotes expression of metastatic factors.[2]

A number of dietary chemopreventive compounds such as flavonoids and curcumin block activation of NFκB.[1,9] Curcumin (Fig. 1) is a non-nutritive, non-toxic compound in turmeric, a spice that has been used for

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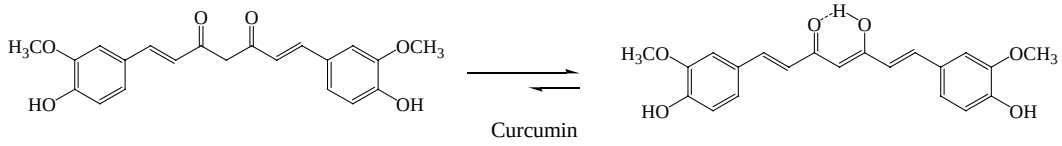


Figure 1. Curcumin exists in solution as an equilibrium mixture of the symmetrical dienone and the keto–enol tautomer stabilized by intramolecular H-bonding.

centuries in India and elsewhere as an herbal medicinal treatment of wounds, jaundice, and rheumatoid arthritis.[10] In addition, curcumin inhibits the proliferation of a variety of tumor cells[9] and has anti-metastatic activity.[11] Curcumin also exhibits potent anti-oxidant activity, which depends upon the presence of phenolic groups in the aryl rings.[12] Previously, we described the anti-oxidant activities of curcumin, as well as enone and dienone analogues of curcumin.[13] In the present study, curcumin and analogues were compared for their abilities to inhibit the TNFα-induced activation of NFκB in cells stably transfected with an NFκB-dependent reporter construct, using the commercially available Panomics' Reporter Stable Cell Line. The role of the enone or dienone functionality in diaryl systems was examined where the spacer is 7-carbons (as in curcumin), 5-carbons or 3-carbons. The role of the aryl ring substituents was also examined. The abilities of the analogues to inhibit the activation of NFκB were compared with their anti-oxidant activities to determine whether both activities derive from general redox chemistry or whether the inhibition of NFκB is selective and unrelated to anti-oxidant activity, which would point toward inhibition of specific targets important in the activation of NFκB.

2. Chemistry

Curcumin is a relatively simple bisphenolic compound that can be prepared in a single reaction. The procedures used to prepare several of the series 1–3 curcumin analogues were described previously.[13] The following schemes summarize the procedures used to prepare new series 1–3 curcumin analogues. For clarity the numbering of the compounds in the schemes correlates to the compound numbers found in Figure 2.

Compounds in series 1 contain a 7-carbon spacer. Five series 1 analogues, **2**, **3**, **15**, **17**, and **20**, were prepared as shown in Scheme 1. Compounds **2**, **3**, **15**, **17**, and **20** contain two aryl rings separated by an unsaturated seven-carbon spacer. Compounds **2**, **3**, **17**, and **20** have either one or two substituents attached to the central methylene carbon, whereas compound **15** is devoid of a central substituent. Compounds **2** and **3** were prepared by reaction of 3-benzyl-2,4-pentanedione or 3-methyl-2,4-pentanedione with 4-hydroxy-3-methoxybenzaldehyde in an aldol-type reaction following the procedure described by Pabon.[14] As expected, in the proton NMR of compounds **2** and **3** the enol form predominates, whereas the carbon spectra exhibit both the keto- and enol-forms. [15] Compounds **15** and **20** were prepared in a similar fashion by reaction of 2,4-pentanedione or 3-methyl-2,4-pentanedione with benzaldehyde. Compound **17** was prepared from compound **15** by reaction with sodium hydroxide and benzyl bromide.[13]

Three additional series 1 curcumin analogues, **4**, **5**, and **6**, were prepared as shown in Scheme 1. Compounds **4**–**6** contain two aryl rings separated by a saturated seven-carbon spacer having two carbonyls. Compounds **4**, **5**, and **6** were prepared by reduction of **3**, **17**, and **20**, respectively, using a procedure described by Venkateswarlu et al.[16] In the proton and carbon NMR's of compounds **4** and **6**, a mixture of the keto- and enol- forms was observed.[17]

Compounds in series 2, which contain a 5-carbon spacer, were prepared as shown in Scheme 1. Compounds **23**, **29**, **34**, **36**, **38**–**41**, and **55** contain two identical aryl rings separated by an unsaturated 5-carbon spacer having a single carbonyl. Compounds **23**, **29**, **34**, **38**, **40**, and **41** were prepared from acetone and a substituted benzaldehyde in a base catalyzed aldol reaction as described by Masuda et al.[18] Compounds **39** and **55** were prepared in a similar fashion by reaction of benzaldehyde or 3,4-dimethoxybenzaldehyde with acetone.[13] Compound **36** was prepared from compound **55** by reaction with boron tribromide following a procedure as described by Royer et al.[19]

Compounds **31** and **44**, which are series 2 analogues, were prepared as shown in Scheme 2. These compounds contain two identical aryl rings separated by a 5-carbon spacer containing both a carbonyl and two epoxide rings. Compounds **31** and **44** were synthesized following the procedure of Yadav and Kapoor[20] by reaction of compound **39** with *tert*-butyl hydroperoxide and aluminum oxide–potassium fluoride. A mixture containing the *trans*/*trans* and *cis*/*cis* isomers was formed and the *trans*/*trans* isomer, compound **31**, was separated from the *cis*/*cis* isomer, compound **44**, through recrystallization from ethanol.[20]

Compound **63**, a series 3 analogue, was prepared as shown in Scheme 2. This compound contains two identical aryl rings separated by a 3-atom spacer having both a carbonyl and a saturated heterocyclic ring. This compound was synthesized following the procedure of Selvaraj et al.[21] by reaction of compound **39** with methylamine. Syntheses of all of the other analogues were described previously.[13]

3. Results

Inhibition of the TNFα-induced activation of NFκB by curcumin and analogues was determined with the Panomics' NFκB Reporter Stable Cell Line 293T/

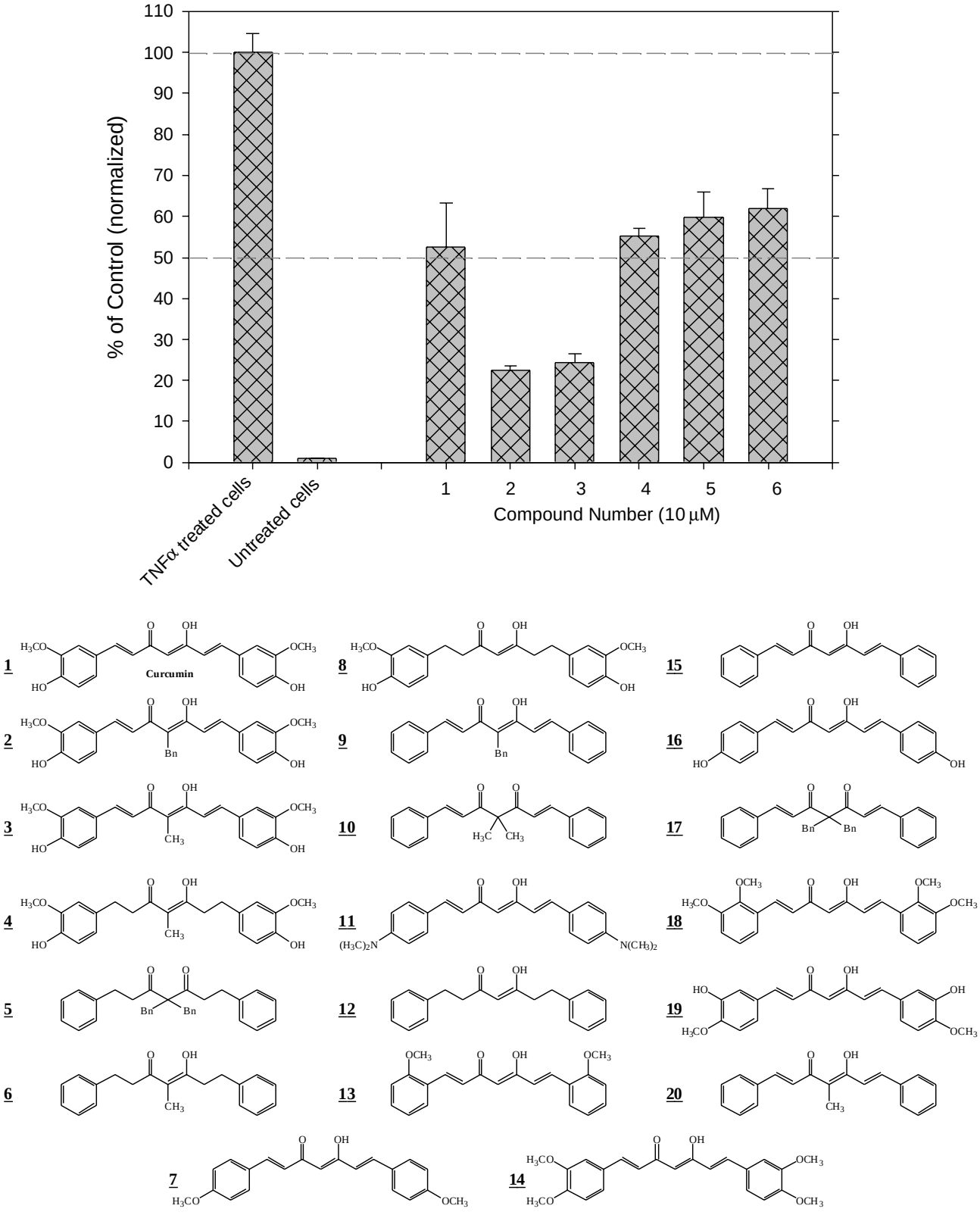
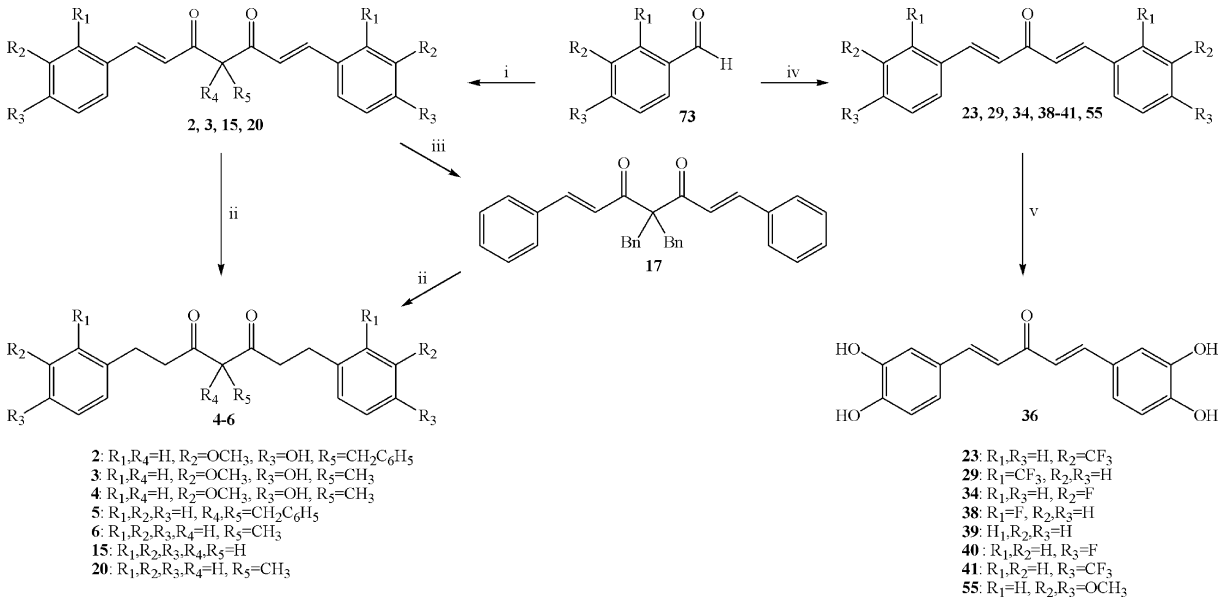
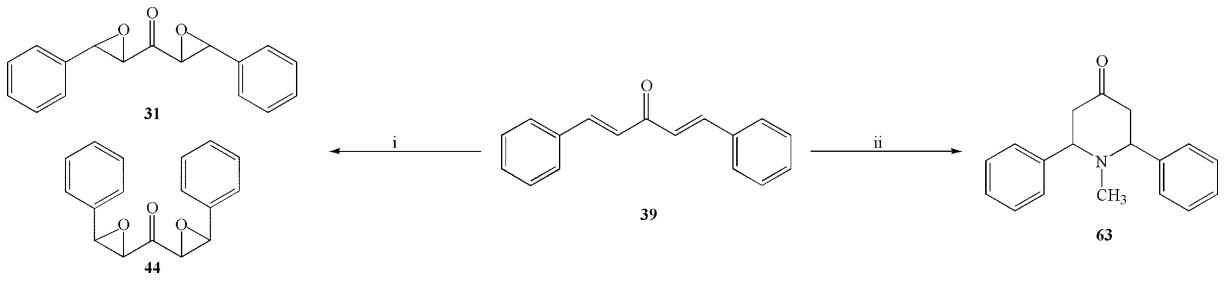


Figure 2. Activities of curcumin (**1**) and analogues in the 7-carbon spacer series as inhibitors of the activation of NFκB by TNFα. Error bars show ±SD. The remaining analogues showed little or no activity.

NFκB-luciferase, which contains a luciferase reporter construct regulated by 6 copies of the NFκB response element. Initially the three series of analogues were screened with curcumin or analogues at 10 μM concentration. All compounds that demonstrated significant activity (26 of the 71 analogues) were then run in triplicate. The results from the analyses of these 26 analogues, compared to curcumin, are summarized in Figures 2–4. The results with curcumin and analogues in series 1 with the 7-carbon spacer are shown in Figure 2.



Scheme 1. Reagents: (i) $CH_3C(O)CH_2C(O)CH_3$ or $CH_3C(O)CH(CH_3)C(O)CH_3$ or $CH_3C(O)CH(CH_2C_6H_5)C(O)CH_3$ (0.5 equiv), B_2O_3, $B(OC_4H_9)_3$, *n*-$BuNH_2$, EtOAc; (ii) H_2, Pd/C, EtOAc; (iii) $C_6H_5CH_2Br$ (2 equiv), NaOH, NH_4Cl, CH_2Cl_2; (iv) $CH_3C(O)CH_3$ (0.5 equiv), NaOH, H_2O, EtOH; (v) BBr_3, CH_2Cl_2, N_2.



Scheme 2. Reagents: (i) CH_3CN, $KF–Al_2O_3$, *t*-BuOOH, $ClCH_2CH_2Cl$; (ii) CH_3NH_2, DMF, H_2O.

Curcumin (**1**) is among the most active members of this series. Analogues **2** and **3**, which contain a single substituent attached to the central methylene carbon but retain the same aryl ring substituents as curcumin, are more active than curcumin. Analogue **4**, the saturated form of analogue **3**, is no longer an enone and is almost as active as curcumin; this suggests that the enone or dienone functionality is not essential for activity. This is further supported by the activities of analogues **5**, **6**, **8**, and **12** that also are devoid of enone functionality. An additional conclusion can be drawn regarding the importance of anti-oxidant activity in the abilities of these analogues to inhibit the activation of NFκB. Analogues **10** and **12** do not exhibit anti-oxidant activity[13] but do inhibit the activation of NFκB.

The results with analogues in series 2 with the 5-carbon spacer are shown in Figure 3. Significantly, many of the analogues are more active than curcumin. This includes enones that are devoid of anti-oxidant activity[13] (**21**, **24**–**26**), further supporting the conclusion that there is no correlation between anti-oxidant activity and inhibition of the activation of NFκB. A number of analogues in this series that are devoid of the enone functionality retain measurable activity (**31**, **42**, and **44**). Nevertheless, the analogues that are more active than curcumin retain the enone functionality.

The results with analogues in series 3 with the 3-carbon spacer are shown in Figure 4. Analogue **61** was more active than curcumin with analogues **62** and **63** exhibiting activities comparable to those of curcumin. These analogues retain the enone functionality and exhibit anti-oxidant activity. Analogue **63**, which is almost as active as curcumin, is devoid of the enone functionality.

The analogues that were more active than curcumin were compared with curcumin in dose–response studies run in triplicate, from which IC_{50} values were obtained. Representative dose–response curves are shown in Figure 5, and IC_{50} values are summarized in Table 1. Also included in Table 1 is reference to whether the active analogues exhibited anti-oxidant activities. The most active analogues exhibit IC_{50} values in the low micromolar range. Analogue **24**, which contains heterocyclic rings, is devoid of anti-oxidant activity, but is the most active against NFκB, with an IC_{50} of 3.4 ± 0.2 μM. In separate experiments, the analogues in Table 1 were examined for cellular toxicity. Cells were treated with analogue, 15 μM, under the same conditions as before but then

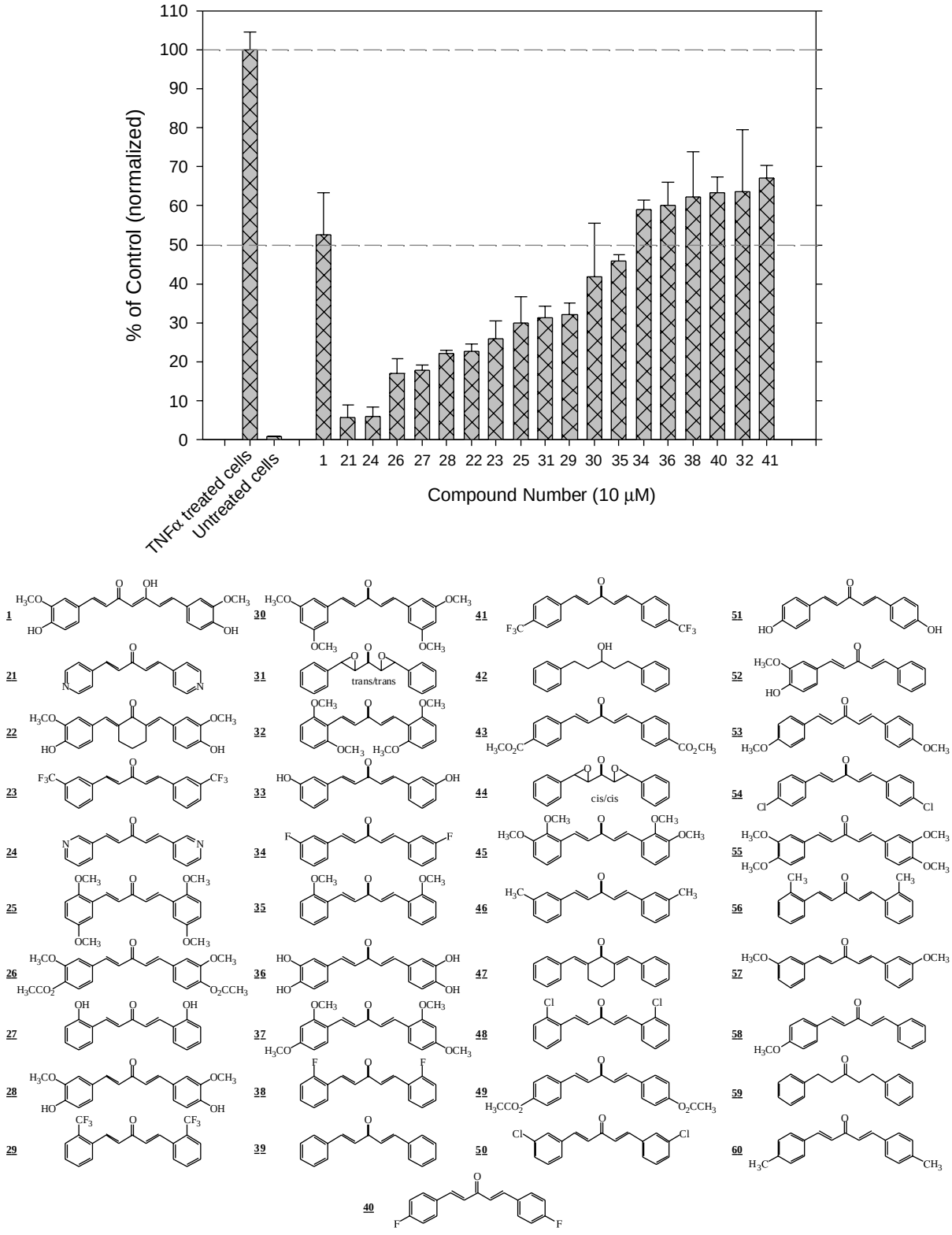


Figure 3. Activities of curcumin (**1**) and analogues in the 5-carbon spacer series as inhibitors of the activation of NFκB by TNFα.

were examined for cell viability rather than for luciferase activity. This concentration was the highest concentration that was generally used in the dose–response studies. Cell viability was determined colorimetrically with the CellTiter 96® AQ_{ueous} One Solution Cell Proliferation Assay (Promega). Cell viability was generally greater than 90%. Therefore, the values listed in Table 1 represent inhibition of the activation of NFκB and are not the result of cell death.

A preliminary QSAR analysis of the data was carried out using the Catalyst program (Accelrys). A wide range of

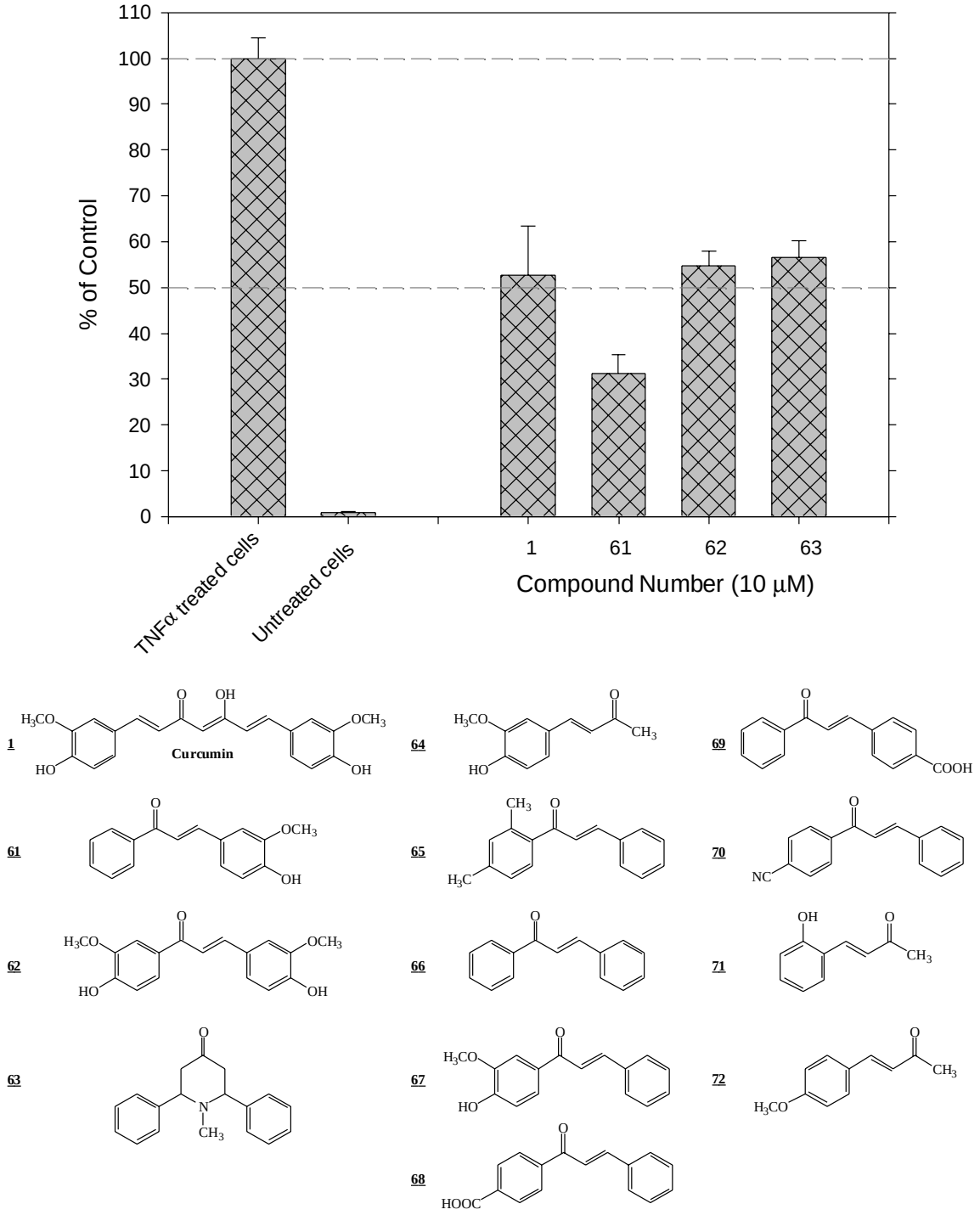


Figure 4. Activities of curcumin (**1**) and analogues in the 3-carbon spacer series as inhibitors of the activation of NFκB by TNFα.

structures and activities from the results in Figures 2–4 were used to generate multiple pharmacophores. A single pharmacophore did not provide a satifactory fit of the data. Moreover, pharmacaphores that were derived separately from 5-carbon curcumin analogues or from 3-carbon analogues did not provide satisfactory fits. However, a single pharmacophore could provide a satisfactory fit of the data for analogues in the 7-carbon series. Figure 6 shows a pharmacophore on which curcumin is superimposed. This pharmacophore provided an excellent fit (correlation 0.9) of analogues on the 7-carbon series. The inability of a single pharmacaphore to provide a satisfactory fit of all of the data or of the data from the 5-carbon series or 3-carbon series may mean that there are several different targets of these analogues.

4. Discussion

Curcumin has a broad range of biological activities, some of which may derive from its anti-oxidant activity or ability to quench free radical reactions and some that

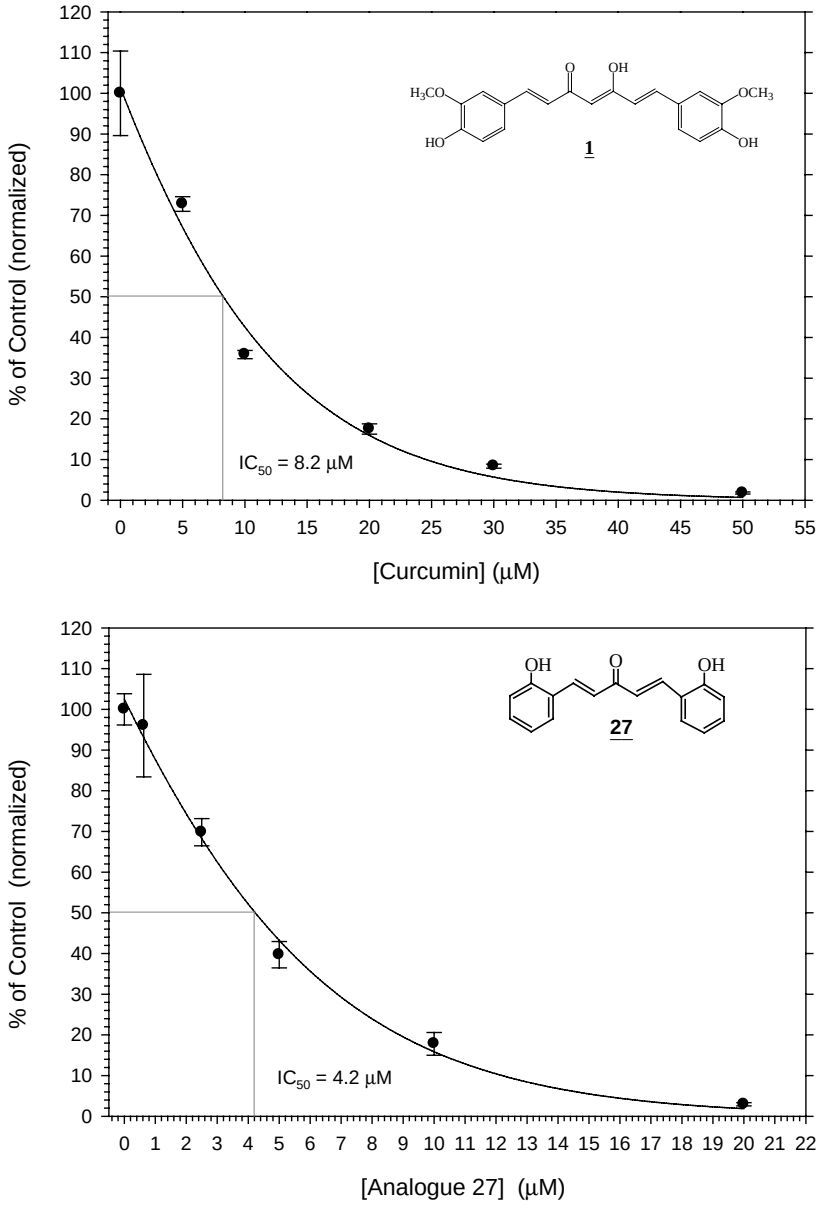
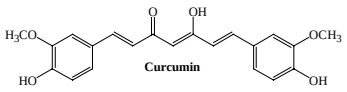
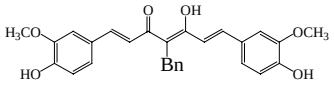
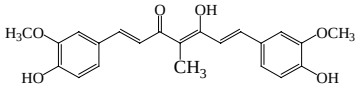
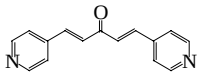
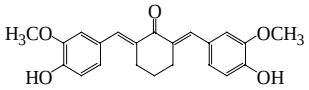
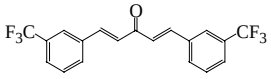
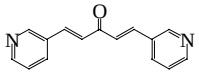
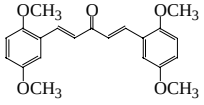
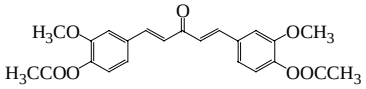
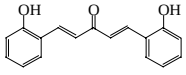
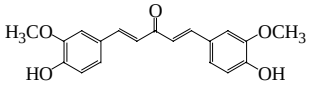
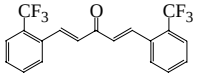
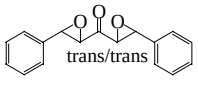
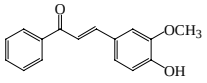


Figure 5. Dose–response curves for curcumin (top) and analogue **27** (bottom) are representative of the dose–response curves for the analogues summarized in Table 1. Curves were generated by Sigma plot's regression wizard (curve fitter).

involve inhibition or inactivation of specific targets. Curcumin can scavenge superoxide radicals, hydrogen peroxide and nitric oxide, and it has been suggested that the ability of curcumin to protect against radiation damage, iron-induced hepatic damage, xanthine oxidase injury, and oxidative stress depends upon the anti-oxidant and free radical-scavenging properties of curcumin.[22–25] In our previous study of the anti-oxidant properties of curcumin,[13] we determined the abilities of curcumin and analogues to quench the pre-formed radical monocation of 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), known as the total radical-trapping anti-oxidant parameter (TRAP) assay, and the abilities of these compounds to reduce the ferric tripyridyltriazine complex, known as the ferric reducing/anti-oxidant power (FRAP) assay.[26] It is noteworthy that many of the most active analogues in the present study (**21**, **23**–**26**) show no activity in the TRAP or FRAP assay, which leads us to conclude that there is no correlation between anti-oxidant activity and ability to inhibit the TNFα-induced activation of NFκB. The lack of correlation between the anti-oxidant activities of curcumin and analogues, and the abilities of these compounds to inhibit the TNFα-induced activation of NFκB suggests that curcumin and analogues inhibit a specific target (or targets) rather than function through general redox chemistry.

The anti-inflammatory properties of curcumin and its ability to inhibit the immune response upon exposure to a variety of external stimuli may, at least in part, result from inhibition of the activation of NFκB by these external signals, since many of the genes that are implicated in the immune/inflammatory response are up-regulated by NFκB. For example, cucumin inhibits the LPS-induced production of IL-1β and TNFα[27], and

Table 1. IC_{50}values for inhibition by curcumin (**1**) and analogues of the TNFα-induced activation of NFκB

Structure	Analogue #	IC_{50}[a] (μM)	*p* values[b]	TRAP[c]	FRAP[d]
 Curcumin	**1**	8.2 ± 0.4			
 Bn	**2**	6.3 ± 0.5	<0.005	+	–
 CH_3	**3**	6.7 ± 1.2	NS[e]	+	+
	**21**	3.9 ± 0.3	<0.001	–	–
	**22**	4.4 ± 0.8	<0.002	+	+
	**23**	7.0 ± 0.5	<0.04	–	–
	**24**	3.4 ± 0.2	<0.001	–	–
	**25**	6.5 ± 0.9	<0.05	–	–
	**26**	5.3 ± 1.1	<0.02	–	–
	**27**	4.2 ± 0.3	<0.001	+	–
	**28**	9.6 ± 0.7	NS	–	–
	**29**	5.0 ± 0.3	<0.001	–	–
 trans/trans	**31**	8.3 ± 1.5	NS	–	–
	**61**	6.2 ± 0.1	<0.001	+	+

[a] IC_{50} values ± standard deviation ($n = 3$).
[b] *t* test, *p* values with curcumin as the reference.
[c] TRAP, total radical-trapping anti-oxidant parameter assay.
[d] FRAP, ferric reducing/anti-oxidant power assay.
[e] NS, not significant.

the IL-1β-induced expression of IL-2,[28] as well as the TNFα-induced expression of ICAM-1, VCAM-1, and E-Selectin.[29] NFκB is implicated in these signaling pathways.[30,31] However, curcumin has also been shown to be a direct inhibitor of enzymes that are important in the inflammatory response, including lipoxygenase and cyclooxygenase.[32]

The extensive literature on the anti-cancer properties of curcumin also includes reports of direct inhibition

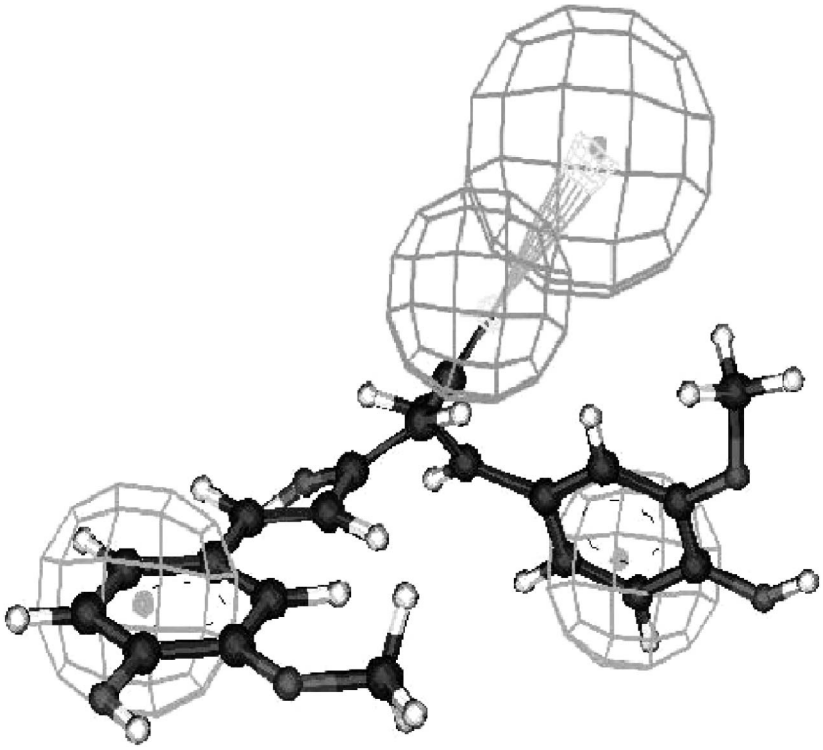


Figure 6. Curcumin aligned with the hypothesis (pharmacophor model) generated with the Catalyst program using compounds **1**–**7** as the training set. The hypothesis consists of two hydrophobic aromatic regions with centers 11.8 angstroms apart and a hydrogen bond acceptor 6.2 Å from the nearest hydrophobic aromatic region and 7 Å from the other. Using this hypothesis, the correlation coefficient for the linear correlation between predicted activity and actual activity was 0.9.

by curcumin of enzymes that may be important in cancer progression, such as inhibition of c-Jun N-terminal kinase,[33] epidermal growth factor receptor[34], and p185neu.[35] The pro-apoptotic activity of curcumin in many types of cancer cells, where the classical hallmarks of apoptosis are observed including DNA laddering, chromatin condensation, and cleavage of 28S and 18S ribosomal RNA,[36] may be related to the activation of NFκB since many cancer cells protect against apoptosis by activating NFκB as a pro-survival strategy. Consequently, much of the interest in the mechanism of curcumin in cancer biology has been directed at NFκB. Over-expression of p65 renders cells resistant to the pro-apoptotic effects of curcumin.[37] When these cells are then transiently transfected with a super-repressor form of IκBα, these cells are no longer resistant to curcumin, consistent with an important role for NFκB in the apoptosis-inducing activity of curcumin. Studies designed to identify the specific target(s) of curcumin in preventing the activation of NFκB point toward targets that are upstream from IκB. Singh and Aggarwal reported that curcumin inhibited TNFα-dependent activation of NFκB at a step before phosphorylation of IκB but after a point where multiple stimuli converge.[38] Brennan and O'Neill reported that curcumin inhibited degradation of IκB but also reacted with p50.[39] Several groups reported that curcumin inhibited IKK,[40,41] or that curcumin targets a kinase that is upstream from IKK.[40] The IKK complex includes IKKα, IKKβ, IKKγ/NEMO, as well as the IκB recruiter/regulator ELKS,[42] and the chaperone HSP90 and co-chaperone Cdc37.[43] More recently, c-Src has been reported to be part of the IKK complex.[44] In addition, NFκB-inducing kinase (NIK), which usually is assigned a role in the alternative activation pathway, has recently been shown to have a role in the activation of IKK.[45] Thus, there are numerous kinases and other proteins that are associated with the IKK complex that are potential targets of the analogues of curcumin.

Recent studies of the biological activities of curcumin and its analogues have focused on the anti-angiogenic properties of these compounds.[46–50] Analogues of curcumin retaining the same aryl ring substituents, including the phenolic groups, were more active than curcumin in their anti-angiogenic activities. Other analogues exhibited anticancer activity against a wide range of cells. An analogue, designated EF24, induces apoptosis in breast and prostate cell lines by a redox-dependent process.[49] ER24 does not contain phenolic groups and is structurally related to our analogues with the 5-carbon spacer. This is consistent with our conclusion that analogues of curcumin may exhibit a range of activities, not all of which are related to the anti-oxidant properties of curcumin.

5. Conclusions

In summary, analogues of curcumin in which the two aryl rings are separated by 7-carbon, 5-carbon or 3-carbon spacers are able to inhibit the TNFα-induced

activation of NFκB. However, activities can vary widely. The most active analogues retain the enone functionality, although this functionality is not essential for activity. In addition, analogues with the 5-carbon spacer are generally the most active. Ring substituents are not necessary but can affect activity. Moreover, the aryl rings can be nitrogen heterocycles. The inhibition of TNFα-induced activation of NFκB by curcumin and analogues may occur at the level of the IKK complex. This is currently being investigated.

6. Experimental

6.1. Chemical synthesis

Melting points were determined with a Thomas-Hoover Uni-Melt apparatus and are uncorrected. NMR spectra were recorded on a Bruker AC250 NMR spectrometer in $CDCl_3$ unless otherwise noted. Chemical shifts are in ppm (δ) relative to TMS. High resolution mass spectra were recorded on a Waters/Micromass LCT-premier. Analytical data were obtained from Galbraith Laboratories, Knoxville, Tennessee. Compounds **2** and **3** were prepared using a modification of Pabon's method.[14] Compounds **4**–**6** were prepared following the method of Venkateswarlu et al.[16] Compounds **23**, **29**, **34**, **38**, **40**, and **41** were prepared following the method of Masuda et al.[18] Compound **36** was prepared following the procedure of Royer et al.[19] Compounds **31** and **44** were prepared following the method of Yadav and Kapoor.[20] Compound **63** was prepared following the procedure of Selvaraj et al.[21] Compounds **1**, **7**–**22**, **24**–**28**, **30**, **32**, **33**, **35**, **37**, **39**, **42**, **43**, **45**–**62**, and **64**–**72** were prepared according to procedures described by Weber et al.[13]

6.2. 4-Benzyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (2)

Yield: 61%. Orange-yellow solid: mp 144–146 °C [lit.[15] 139–141 °C]; ^{1}H NMR δ (DMSO) 3.81 (s, 6H), 4.11 (s, 2H), 6.78 (d, 2H, J = 8.1 Hz), 7.10–7.30 (m, 11H), 7.58 (d, 2H, J = 15.1 Hz); ^{13}C NMR: δ 30.2, 33.7, 55.6, 55.7, 63.0, 109.9, 111.3, 115.5, 117.9, 122.4, 123.2, 123.7, 125.5, 125.7, 126.0, 126.5, 127.7, 128.1, 128.3, 128.7, 139.1, 141.7, 142.3, 144.1, 147.8, 149.2, 149.7, 183.0, 194.0; Exact Mass Calcd for $C_{28}H_{26}O_6$: 458.1729, obsd (M+H) 459.1798.

6.3. 4-Methyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (3)

Prepared according to the method of Pabon[14] by reaction of 3-methyl-2,4-pentanedione (1.14 g, 10 mmol) and 4-hydroxy-3-methoxybenzaldehyde (3.04 g, 20 mmol) to give 1.08 g (28%) of an orange solid: mp 180–183 °C; ^{1}H NMR (DMSO) δ 2.20 (s, 3H), 3.84 (s, 6H), 6.81 (d, 2H, J = 8.14 Hz), 7.17 (d, 2H, J = 15.7 Hz), 7.19 (d, 2H, J = 7.2 Hz), 7.57 (d, 2H, J = 15.3 Hz), 9.62 (s, 2H); ^{13}C NMR: δ 11.5, 13.1, 55.2, 55.6, 55.8, 105.6, 111.4, 111.6, 115.5, 117.7, 122.1, 123.2, 123.5, 125.6, 126.6, 141.4, 143.7, 147.8, 149.1, 149.6, 182.1, 196.0. Anal. Calcd for $C_{22}H_{22}O_6$: C, 69.10; H, 5.80. Found: C, 69.19; H, 5.89.

6.4. 4-Methyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-heptane-3,5- dione (4)

Prepared according to the method of Venkateswarlu et al.[16] by reaction of compound **3** (0.20 g, 0.5 mmol) with 10% palladium on activated carbon (25 mg) under a hydrogen atmosphere at 60 psi to give 0.10 g (48%) of a pale yellow oil: ^{1}H NMR δ 1.23 (d, 3H, J = 7.2 Hz), 1.69 (s, 3H), 2.72 (m, 16H), 3.57 (q, 1H, J = 7.0 Hz), 3.83 (s, 12H), 5.48 (s, 4H), 6.58–6.85 (m, 12H); ^{13}C NMR: δ 12.5, 29.2, 30.6, 43.3, 55.9, 61.4, 110.9, 111.0, 114.2, 114.3, 120.7, 132.4, 132.6, 143.9, 146.3, 206.1; Exact Mass Calcd for $C_{22}H_{26}O_6$: 386.1729, obsd (M+H) 387.1783.

6.5. 4,4-Dibenzyl-1,7-diphenylheptane-3,5-dione (5)

Prepared according to the method of Venkateswarlu et al.[16] by reaction of compound **17** (70 mg, 0.15 mmol) with 10% palladium on activated carbon (10 mg) under a hydrogen atmosphere at 60 psi to give 60 mg (86%) of a white solid: mp 100.5–101.5 °C; ^{1}H NMR δ 2.56 (t, 4H, J = 7.4 Hz), 2.74 (t, 4H, J = 6.8 Hz), 3.29 (s, 4H), 6.88–7.20 (m, 20H); ^{13}C NMR: δ 29.6, 37.3, 42.5, 71.1, 126.1, 126.8, 128.4, 129.6, 136.0, 140.6, 207.8. Anal. Calcd for $C_{33}H_{32}O_2$: C, 86.05; H, 7.00. Found: C, 86.28; H, 7.11.

6.6. 4-Methyl-1,7-diphenylheptane-3,5-dione (6)

Prepared according to the method of Venkateswarlu et al.[16] by reaction of compound **20** (0.96 g, 3.3 mmol) with 10% palladium on activated carbon (25 mg) under a hydrogen atmosphere at 60 psi to give 0.71 g (73%) of a clear oil: ^{1}H NMR δ 1.20 (d, 3H, J = 7.2 Hz), 1.66 (s, 3H), 2.61–2.85 (m, 16H), 3.55 (q, 1H, J = 7.0 Hz), 7.08–7.25 (m, 20H); ^{13}C NMR: δ 12.3, 29.3, 31.0, 37.6, 42.8, 60.9, 104.2, 125.9, 128.1, 128.2, 140.4, 140.8, 174.6, 191.5, 205.6; Exact Mass Calcd for $C_{20}H_{22}O_2$: 294.1620, obsd (M+H) 295.1693.

6.7. 1,7-Diphenyl-1,6-heptadiene-3,5-dione (15)

Yield: 38%. Yellow solid: mp 140–142 °C [lit.[13,15] 139–140 °C].

6.8. 4,4-Dibenzyl-1,7-diphenyl-1,6-heptadiene-3,5-dione (17)

Yield: 75%. White solid: mp 182–183 °C [lit.[13,15] 181 °C].

6.9. 4-Methyl-1,7-diphenyl-1,6-heptadiene-3,5-dione (20)

Yield: 59%. Orange solid: mp 154–157 °C [lit.[13] 154–157 °C].

6.10. 1,5-Bis-(3-trifluoromethylphenyl)-1,4-pentadien-3-one (23)

Prepared according to the method of Masuda et al.[18] by reaction of α,α,α-trifluoro-*m*-tolualdehyde (0.5 ml, 3.8 mmol) and acetone (0.14 ml, 1.9 mmol) to give 0.25 g (36%) of yellow crystals: mp 116–117 °C; ^{1}H NMR: δ 7.12 (d, 2H, J = 15.9 Hz), 7.72 (m, 10H); ^{13}C

NMR: δ 123.7, 124.7, 126.7, 126.8, 129.5, 131.5, 131.6, 135.4, 141.8, 187.8. Anal. Calcd for $C_{19}H_{12}OF_6$: C, 61.63; H, 3.27. Found: C, 61.82; H, 3.28.

6.11. 1,5-Bis-(2-trifluoromethylphenyl)-1,4-pentadien-3-one (29)

Yield: 88%. Yellow solid: mp 131–133 °C [lit.[51] 131 °C].

6.12. *trans*, *trans*-1,2,4,5-Diepoxy-1,5-diphenylpentan-3-one (31) and *cis*,*cis*-1,2,4,5-diepoxy-1,5-diphenylpentan-3-one (44)

Prepared according to the method Yadav and Kapoor[20] by reaction of compound **39** (0.47 g, 2.0 mmol) with *tert*-butyl hydroperoxide (1.90 g, 21 mmol) and aluminum oxide–potassium fluoride (0.48 g) to give a mixture of *trans*/*trans* and *cis*/*cis* isomers. Recrystallization from ethanol gave 0.21 g (40%) of the *trans*/*trans* isomer as white crystals: mp 117–119 °C (lit.[52] 118–118.5 °C). Evaporation of the filtrate gave 0.25 g (47%) of the *cis*/*cis* isomer as an orange-yellow oil: ^{1}H NMR: δ 3.71 (d, 2H, J = 1.6 Hz), 4.12 (d, 2H, J = 1.6 Hz), 7.28–7.35 (m, 10H); ^{13}C NMR: δ 58.8, 60.3, 125.8, 128.6, 129.1, 134.4,199.0.

6.13. 1,5-Bis-(3-fluorophenyl)-1,4-pentadien-3-one (34)

Prepared according to the method of Masuda et al.[18] by reaction of 3-fluorobenzaldehyde (0.5 ml, 4.8 mmol) and acetone (0.17 ml, 2.4 mmol) to give 0.26 g (41%) of yellow crystals: mp 96–97 °C; ^{1}H NMR: δ 7.03 (d, 2H, J = 16.1 Hz), 7.09 (m, 2H), 7.35 (m, 6H), 7.67 (d, 2H), J = 15.9 Hz); ^{13}C NMR: δ 114.4, 117.5, 124.4, 126.3, 130.4, 136.9, 142.0, 162.9, 188.1. Anal. Calcd for $C_{17}H_{12}OF_2$: C, 75.55; H, 4.48. Found: C, 75.26; H, 4.65.

6.14. 1,5-Bis-(3,4-dihydroxyphenyl)-1,4-pentadien-3-one (36)

Yield: 76%. Orange solid: mp >250 °C [lit.[53] 221–223 °C]; ^{1}H NMR: δ (DMSO) 6.78 (d, 2H, J = 8.1 Hz), 6.99 (d, 2H, J = 15.9 Hz), 7.06 (d, 2H, J = 7.9 Hz), 7.14 (s, 2H), 7.55 (d, 2H, J = 15.7 Hz), 9.15 (s, 2H), 9.63 (s, 2H); ^{13}C NMR: δ 114.9, 115.6, 121.5, 122.5, 126.2, 142.5, 145.4, 148.2, 187.6.

6.15. 1,5-Bis-(2-fluorophenyl)-1,4-pentadien-3-one (38)

Yield: 42%. Yellow crystals: mp 68–71 °C [lit.[49] 68–70 °C].

6.16. 1,5-Diphenyl-1,4-pentadien-3-one (39)

Yield: 86%. Yellow crystals: mp 110–112 °C [lit.[13,54] 112–114 °C].

6.17. 1,5-Bis-(4-fluorophenyl)-1,4-pentadien-3-one (40)

Yield: 87%. Yellow solid: mp 150–152 °C [lit.[55] 152–154 °C]; ^{13}C NMR: δ 116.1, 1125.1, 130.2, 130.9, 142.0, 164.0, 188.3.

6.18. 1,5-Bis-(4-trifluoromethylphenyl)-1,4-pentadien-3-one (41)

Yield: 84%. Yellow solid: mp 152–154 °C [lit.[56] 156–157 °C]; ^{1}H NMR: δ 7.12 (d, 2H, J = 15.9 Hz), 7.69 (m, 10H); ^{13}C NMR: δ 121.6, 125.9, 127.2, 128.5, 132.1, 138.0, 141.8, 187.9.

6.19. 1,5-Bis(3,4-dimethoxyphenyl)-1,4-pentadien-3-one (55)

Yield: 75%. Yellow solid: mp 72–75 °C [lit.[13,53] 68–70 °C].

6.20. 1-Methyl-2,6-diphenyl-4-piperidone (63)

Yield: 42%. White solid: mp 147–149 °C [lit.[21] 147–149 °C].

6.21. Cell assay

An NFκB reporter stable cell line from human 293T embryonic kidney cells (293T/NFκB-luc) (Panomics, Inc., Redwood City, CA) was grown in a humidified atmosphere at 37 °C in 5% CO_2/95% air. The cells were maintained in Dulbecco's modified Eagle's medium (DMEM—high glucose containing 4 mM glutamine) supplemented with 10% fetal bovine serum (FBS), 1 mM sodium pyruvate, 100 U/ml penicillin, 100 μg/ml streptomycin, and 100 μg/ml hygromycin (Gibco/Invitrogen, Carlsbad, CA) to maintain cell selection.

One day prior to treatment, the 293T/NFκB-luc cells were plated into 24-well cell culture plates (Costar, Cambridge, MA) at approximately 70% confluency in the above media without hygromycin. The following day cells were fed fresh media 1 h prior to treatment. Media with or without recombinant tumor necrosis factor alpha (TNFα) (R&D Biosciences/Clontech, Palo Alto, CA) were then applied to the cells at 20 ng/ml followed by immediate treatments with curcumin or analogue. The cells were placed again in a humidified atmosphere at 37 °C in 5% CO_2/95% air for 7 h. Plate wells were gently washed with phosphate-buffered saline, pH 7.4, and lysed with 1× passive lysis buffer (Promega, Madison, WI). The subsequent lysates were analyzed with the Luciferase Assay System (Promega) utilizing a TD-20/20 luminometer (Turner Designs, Sunnyvale, CA). The firefly luciferase relative light units were normalized to protein (mg/ml) with BCA™ Protein Assay Kit (Pierce, Rockford, IL) and standardized to percent of control (TNFα control). For assays of cell viability, cells were treated similarly as above and with 15 μM analogue. After washing, cells were treated with 100 μl media and 20 μl CellTiter 96® AQ_{ueous} One Solution reagent for 1 h and then read at 490 nm with a Spectromax plate reader.

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