Bioorganic Chemistry 40 (2012) 30–38

Contents lists available at SciVerse ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis and anti-inflammatory properties of some aromatic and heterocyclic aromatic curcuminoids

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ARTICLE INFO

Article history:
Received 20 May 2011
Available online 25 November 2011

Keywords:
Anti-inflammatory
Curcumin
Curcuminoids
Heterocyclic
Ascorbic acid
In vivo assays
Organic synthesis
Spectroscopy

ABSTRACT

A variety of novel aromatic and heterocyclic aromatic curcuminoids were synthesised, characterised and their anti-inflammatory activities (AIA) determined *in vivo*. Some of these compounds also were tested for inflammatory mediator production. The AIA of the main representatives of these compounds were assessed by oral administration to female Wistar rats using (a) acute carrageenan-induced paw oedema, (b) chronic adjuvant arthritis (therapeutic mode), and (c) anti-pyretic activity assessed in the yeast pyrexia. Gastric ulceration was determined in pre-inflamed rats. Natural curcumin showed modest aspirin-like anti-inflammatory activity which was enhanced when co-administered with the PGE_1 analogue misoprostol as a synergist. In contrast, four novel curcuminoids (RK-97, RK-103, RK-104 and RK-106) in which the *bis*-methoxy-phenyl group of curcumin was replaced with *bis*-dimethoxybutenolidyl-(ascorbate), *bis*-naphthyl, and *bis*-furanyl derivatives, respectively, had potent activity in the anti-arthritic assay with little gastric or systemic toxicity, compared with the vehicle-treated controls. Of the curcuminoids the furan RK-106 was the only compound to inhibit production of TNFα and IL-1β in a monocytic cell-line THP-1 *in vitro*. The inactivity of RK-106 on the production of PGE_2 may be related to its absence of gastrotoxicity. None of the curcuminoids exhibited anti-pyretic activity and this may also be related to its insensitivity to PGE_2. Thus, these novel curcuminoids, such as RK-106, may warrant the development of new low gastro-toxic anti-inflammatory agents with selective inhibitory activity of cytokine inflammatory mediators.

1. Introduction

Curcumin (1) is the yellow pigment and main biologically active component of turmeric, a spice that comes from the *Curcuma longa* rhizome (*Zingaberaceae*) [1,2]. Tumeric has been long used as a seasoning ingredient, the main ingredient in Indian curry, and for its diverse therapeutic activities such as, inflammation and cancer [1–8]. Tumeric contains approximately 5% curcumin and two other compounds demethoxycurcumin (DMC), and bisdemethoxycurcumin (BDMC) [5]. The metabolism of curcumin involves reduction to tetrahydrocurcumin (THC), hexahydrocurcumin (HHC) and hexahydrocurcuminol (HCC) with demethylation and conjugation with glucuronic acid and sulphate [1].

The anti-inflammatory actions of curcumins include anti-oxidant activity, inhibition of signal transduction via NFκB and AP-1, inhibition of phospholipase A_2, cyclo-oxygenases (COX), lipoxygenases (LOX) involved in leukotriene synthesis and nitric oxide [7,9–16]. These activities may be potentially important for the control of osteoarthritis (OA) [17] and pro-apoptotic and growth inhibitory effect in OA synoviocytes [18].

Curcumin and its synthetic derivatives (curcuminoids) have been investigated for their anti-inflammatory and anti-arthritic activities [5,6,10,11,13,19,20]. Amongst these THC, HHC, HCC and some demethoxy- and diacetyl-derivatives of curcumin have been investigated [5,6,10,11,13,19]. Except THC, these derivatives show variable *in vivo* anti-inflammatory activities but diminished inhibitory activity than curcumin [5,6]. One of the studies has reported that an Ar-*para* hydroxyl group is essential for anti-inflammatory activity of curcumin [5]. Many of the reported curcuminoids have simple ring substitutions (e.g. diacetyl groups) or without methyl, methoxy group or hexahydro-reductions of the diethylene components. To our knowledge the effects on anti-inflammatory activity of substitution of benzene rings with furans, thiophenes, naphthyl, or ascorbate derivatives of curcumin have not been previously reported.

The aim of this work was to synthesise a number of curcuminoids as well as some variants of substituted methoxy- or demethoxy-derivatives of curcumin. Selected biological activities of these compounds are also being reported (Tables 1–3).

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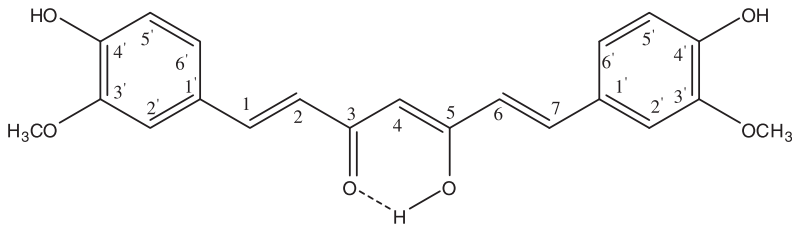
doi:10.1016/j.bioorg.2011.11.004



2. Results and discussion

2.1. Chemistry

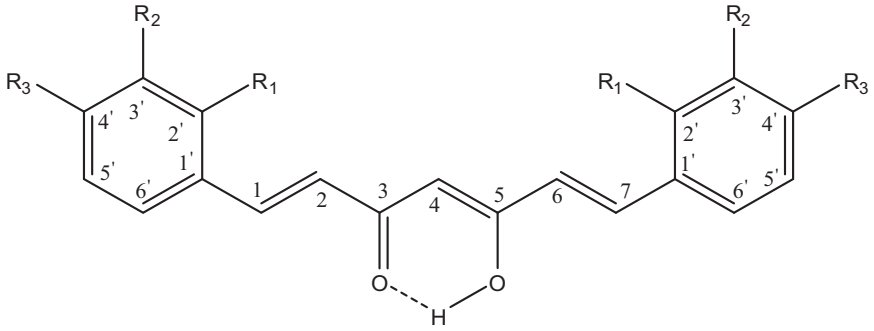
Synthesis of curcumin was first reported by Lampe and coworkers in 1913 [21] and later a modified procedure was published by Pabon [22] and Pederson et al. [23,24]. All our curcuminoids were synthesised by Pabon procedure involving a reaction between the appropriate aldehyde and acetylacetone–boric anhydride complex as shown in Scheme1[25]. In this reaction the active α-methylene group in acetylacetone is protected in the form of an enolate complex with boron which consequently allows the desired condensation reaction to occur between the free methyl groups of acetylacetone and the appropriate aldehyde.

The compounds RK78, RK 87 [5,25], RK 88-RK 91 [5], RK 92 [25,26], RK 107 [20], RK 99 and RK 106 [26] have already been reported in the literature and we have synthesised them by Pabon method [25]. All the synthesised curcuminoids were characterised spectroscopically using ^{1}H and ^{13}C nuclear magnetic resonance and mass spectrometry data. The proton magnetic resonance spectra indicated that all the curcuminoids existed entirely in the keto-enol form rather than the diketo form in deuterochloroform solution. This was supported by the fact that the H NMR spectra of RK 97 and RK 103 showed an additional one proton singlet resonating at δ 7.27 at room temperature which we interpreted as enolic proton resonance.

In all of the mass spectra for the curcuminoids the molecular ion was evident and its accurate mass was measured, Tables 1–3. The prominent fragmentation, observed in all cases is shown in Fig. 1. The prominent ion at m/z $[Ar–CH{=}CH–CO]^{+}$ resulting from molecular cleavage in EI mode was observed as base peak. Many of these curcuminoids showed the same fragmentation forming the base peak which could be used as a marker ion for the identification of curcuminoids structures.

Curcuminoid RK 97 involved an eight-step synthesis starting from L-ascorbic acid [27,28] (Fig. 2). The twofold rationale behind the synthesis of L-ascorbic acid derivative RK97 was based on the recognition that the anti-oxidant activity of L-ascorbic acid is confined to the ene-diol system [28] and secondly ascorbic acid would enhance the water solubility of the curcuminoid molecule possibly enhancing its bioavailability. It is known that curcumin is poorly absorbed in the biosystem and therefore its water soluble ascorbate derivative may be an added advantage for its absorption [29]. The RK 97 is a pro-drug with an ene-diol system in its methyl ether form which *in vivo* could degrade to the free 1,2-dihydroxy system.

Table 1
Physical data of substituted aromatic curcuminoids.

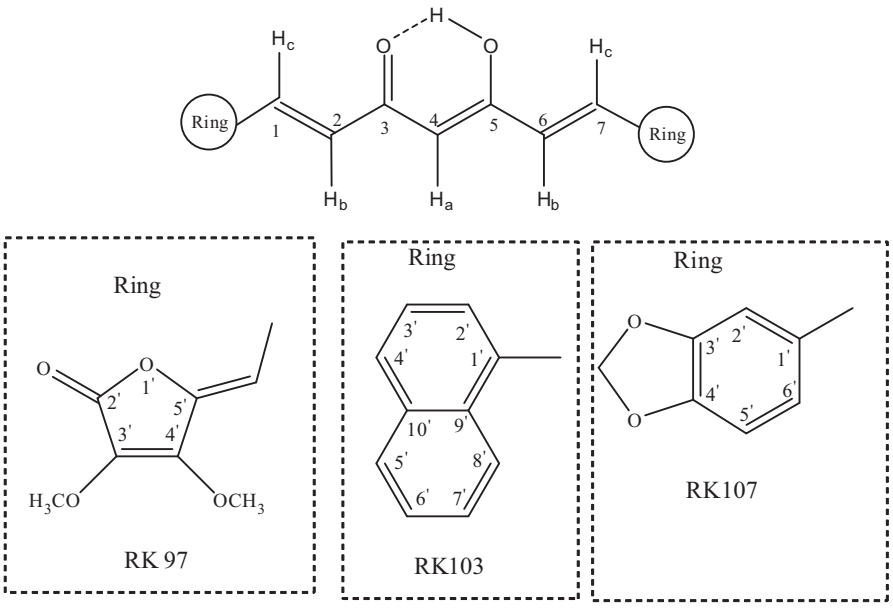


1,7-bis(aryl)-1,6-hepadiene-3,5-dione (enol form)

Code RK	R_1	R_2	R_3	Yield (%)	R_f (solvent)[h]	Mp (°C)	Formula	High resolution MS (M^+) Found (calculated)
78	H	H	H	78.3	0.86	138–140[a]	$C_{19}H_{16}O_2$	276.1142 (276.1150)
87	H	H	OCH_3	57.6	0.62	164–167[b,c]	$C_{21}H_{20}O_4$	336.1336 (336.1362)
88	OCH_3	H	H	58.3	0.64	110–112[d]	$C_{21}H_{20}O_4$	336.1368 (336.1362)
89	H	OCH_3	OCH_3	59	0.12	134–136[e]	$C_{23}H_{24}O_6$	396.1564 (396.1573)
90	H	H	CH_3	73	0.80	198–202[f]	$C_{21}H_{20}O_2$	304.1466 (304.1463)
91	H	OCH_3	H	37	0.62	Gum[g]	$C_{21}H_{20}O_4$	336.1347 (336.1362)

[a] Lit 140–142 °C [3].
[b] Lit154–155 °C [3].
[c] Lit 164–165 °C [12].
[d] Lit 121–122 °C [3].
[e] Lit 128–130 °C [3].
[f] Lit 205–206 °C [3].
[g] Lit 72–74 °C [3].
[h] Ethyl acetate:petroleum ether, (1:2).

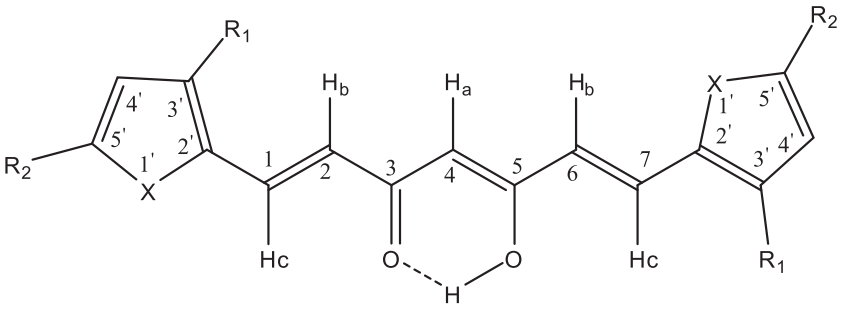
Table 2
Physical data for additional curcuminoids.



Code RK	Yield (%)	R_f (solvent)[a]	Mp (°C)	Formula	High resolution MS (M^+) Found (calculated)
97	50	0.30	>200 dec.	$C_{21}H_{20}O_{10}$	432.1048 (432.1058)
103	79	0.83	167–169	$C_{27}H_{20}O_2$	376.1453 (376.1464)
107	61.5	0.82	193–195	$C_{21}H_{16}O_6$	364.0943 (364.0948)

[a] Ethyl acetate:petrol (1:2).

Table 3
Physical data of heterocyclic aromatic curcuminoids.



1,7-bis(heterocyclyl)-1,6-heptadiene-3,5-dione (enol form)

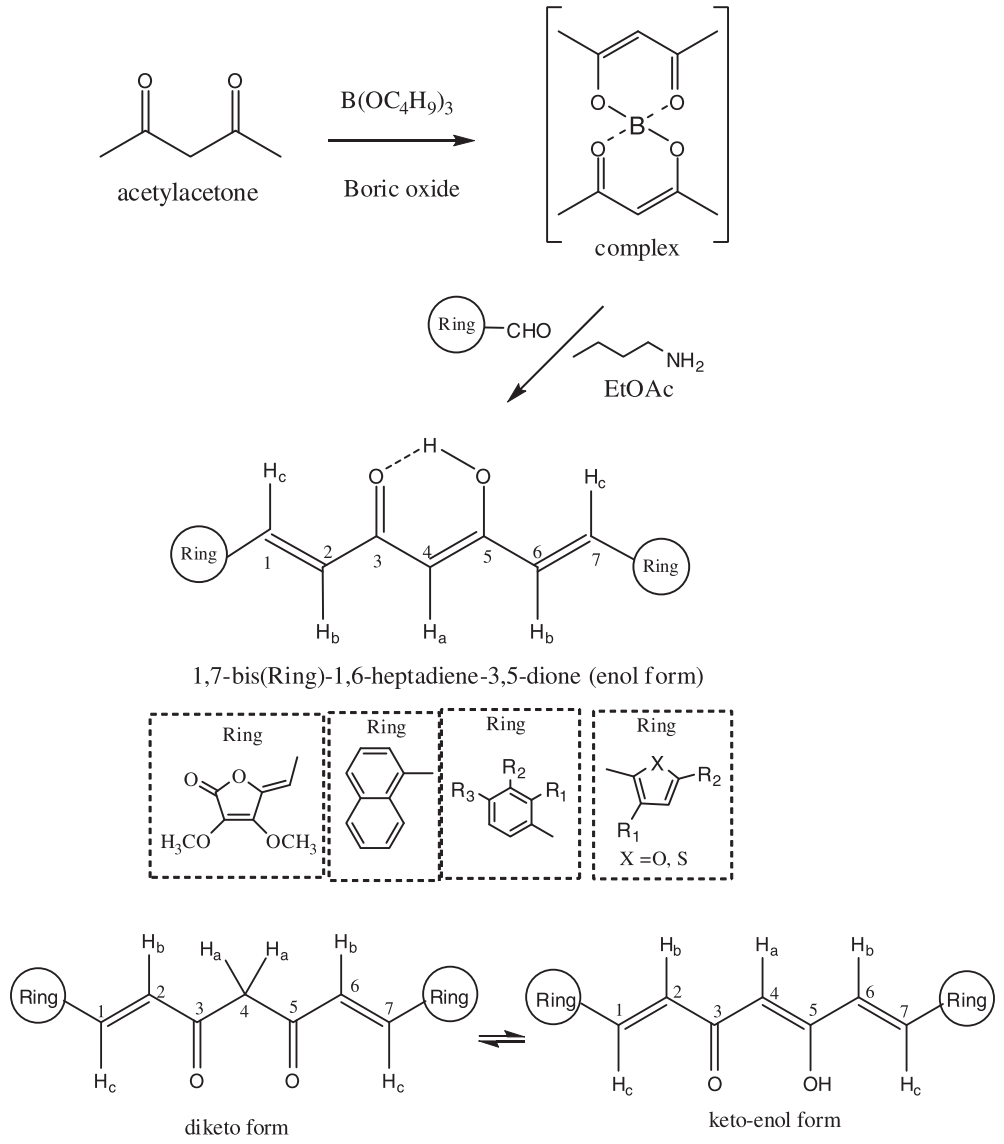
Code RK	R_1	R_2	X	Yield (%)	R_f (solvent)[a]	Mp (°C)	Formula	High resolution MS (M^+) Found (calculated)
92	H	H	O	26	0.73	Oil	$C_{15}H_{12}O_4$	256.0739 (256.0736)
99	H	H	S	28	0.81	184–186	$C_{15}H_{12}O_2S_2$	288.0278 (288.0279)
104	H	CH_3	S	40	0.83	130–132	$C_{17}H_{16}O_2S_2$	316.0594 (316.0592)
105	CH_3	H	S	12	0.85	109–111	$C_{17}H_{16}O_2S_2$	316.0605 (316.0592)
106	H	CH_3	O	63	0.83	144–146	$C_{17}H_{16}O_4$	284.1044 (284.1049)

[a] Ethyl acetate:petroleum (1:2).

2.2. Pharmacology

2.2.1. In vivo activities

In the acute anti-inflammatory assays in carrageenan hind paw oedema compounds RK-92, AK-99 and RK-97 showed inhibitory activity in order of decreasing potency (Table 4). Paradoxically, RK-104 and RK-107 showed enhanced paw swelling indicating their proinflammatory activity. This observation is a feature of some disease-modifying anti-rheumatic drugs (DMARDs) such as levamisole and gold salts [30,31]. The time-course of inflammation is shown in Fig. 3. The most potent anti-inflammatory activity of RK92 and RK 99 was observed at 1 and 2 h after induction of inflammation. Curcumin itself showed minimal nonsteroidal anti-inflammatory drug (NSAID) activity unless co-administered with the PGE_1 analogue, misoprostol, as a synergist [32]. The use of misoprostol was intended to replicate conditions where inflam-



Scheme 1. Synthesis of curcuminoids from acetylacetone by the Pabon method [21].

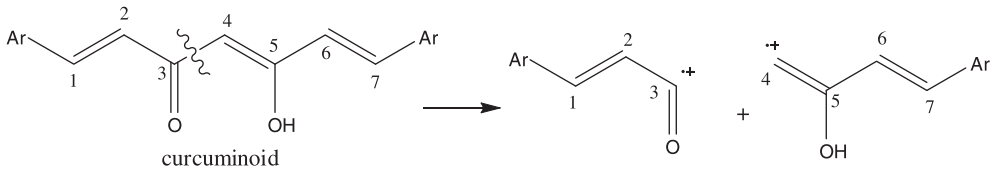


Fig. 1. Synthesis of RK 97 from L-ascorbic acid.

mation could be induced with enhanced anti-inflammatory activity of the drug.

Curcumin did not show anti-pyretic activity at doses up to 300 mg/kg, in contrast to some phenols e.g. paracetamol, salicylic acid/alcohol/amide and some enolic drugs (phenylbutazone, piroxicam) which rapidly reduced pre-established fever within 60 min after oral administration.

At 24 h after oral administration many of the curcuminoids as well as aspirin showed a rebound in hindpaw inflammationwhich is a typical effect of anti-inflammatory agents.

By contrast, the most potent analogues in the chronic anti-arthritic assay were RK-103 and AK-104, followed by RK-107, RK-97 as well as curcumin itself (Table 4). These compounds showed little immediate toxicity, evidenced by normal weight gain and improved physical condition of the animals, compared with the vehicle-treated controls. After withdrawal of the drug a slow rebound of arthritis was observed indicating that the treated animals had responded to the arthritigen, i.e. this was not a false-positive result for the applied test compounds.

In contrast to aspirin and some enolic anti-inflammatory drugs (phenylbutazone, piroxicam), none of these curcuminoids caused gastric irritation sufficient to induce local bleeding into the gastric mucosa (data not shown).

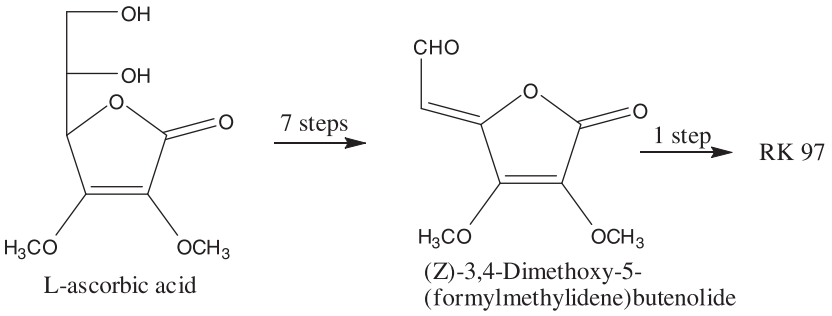


Fig. 2. Synthesis of RK 97 from L-ascorbic acid.

Table 4
Anti-inflammatory activities of some curcuminoids in female Wistar rats.

Compound	Dose mg/kg p.o.	Anti-oedemic activity[a]		Dose mg/kg p.o.	Anti-arthritic activity[b]			
		1 h	2 h		Arthritis score	Rear paw swelling	Fore paw inflamm.	ΔWt gm
RK78	100	39%	37%					
RK88				40	32%	18%	17%	+11
RK89	100	12	45	100	10	36	28	+10
RK92	100	51	55	100	40	32	28	+06
RK97	100	37	37	100	55	103	80	+08
RK99	100	41	52	100	05	08	0	+11
RK103	100	17	0	100	78	70	30	+17
RK104	100	−75[c]	−55	100	78	85	84	+12
RK-106	100	−24	14	100	37	27	21	+15
RK107	100	−61	−36	100	65	52	72	+17
Curcumin (Cmn)	100	18	32	100	75	79	36	+13
Cmn and MPL[d]	100 and 0.6	61	64					
Aspirin	150	44	62					
Phenylbutazone	80	24	36					

Data = mean percentage inhibition of signs of inflammation, compared to vehicle-treated control animals ($n \leqslant 4$).
[a] Measured in the carrageenan paw assay.
[b] Measured in animals with pre-established adjuvant-arthritis at time of early onset and compared with untreated animals (see below).
[c] Negative data indicate increase in paw oedema compared to controls.
[d] MPL = Misoprostol co-administered as a synergist; this dose (0.6 mg/kg) alone being inactive.

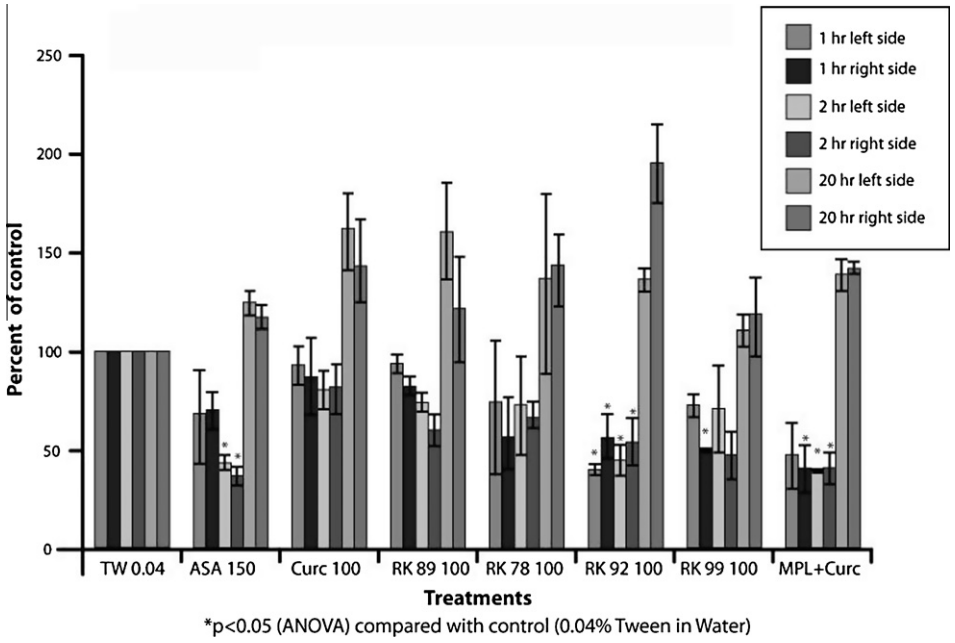


Fig. 3. Effects of curcumin and its derivatives on carrageen – an-induced paw swelling in rats. Values are mean ± SEM.

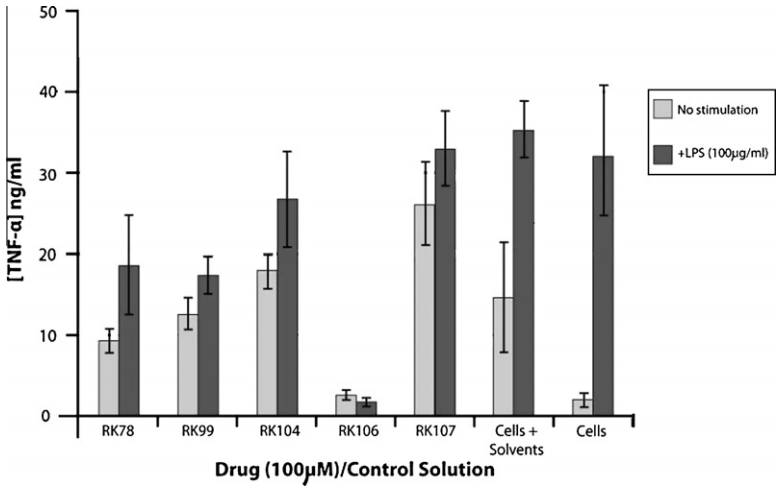


Fig. 4. TNF-α production in curcumin treated THP-1 monocytes ($n = 4$).

2.2.2. Reference data and in vivo inflammatory mediators

Untreated controls ($n = 12$) had mean arthritis score (AS) = 2.7+ (SE 0.2+), mean rear paw swelling = 1.13 mm (SE 0.17) and mean forepaw inflammation index (FI) = 2.5+ (SE = 0.31+). Normal rat rear paws = 7.24 (SE 0.12) mm with zero AS and mean FI $\leqslant$ 0.2+. [AS and FI were scored for each animal on a scale 0 to 4+].

Plasma concentrations of PGE_2 and IL-1β were unaltered in arthritic rats that were orally given curcumin and the other curcuminoids (data not shown). However, plasma levels of PGE_2 were reduced in the plasma of rats treated with aspirin (data not shown) thus showing positive effects of this NSAID in the arthritic animals.

2.2.3. In vitro production of inflammatory mediators

Of the curcuminoids assayed in THP-1 monocytic cells, only RK-106 inhibited production of basal and LPS stimulated TNFα (Fig. 4). This curcuminoid also inhibited LPS-stimulated, but not basal production of IL-1β (data not shown). None of the compounds affected production of PGE_2, even though NSAIDs were effective in reducing production of PGE_2 in the cell line used in this study [37].

3. Materials and methods

3.1. Chemistry

Melting points were recorded on Stuart SMP3 digital apparatus; ^{1}H NMR spectra were recorded on a Brucker AC 250 MHz and ^{13}C NMR spectra were recorded on a Brucker Avance III 400 MHz spectrometers. Mass spectra (MS) were obtained on VG 770E spectrometer in EI mode at 70 eV. TLC analyses were done using pre-coated Merck silica gel-coated aluminium sheets and flash chromatography was performed on BDH flash silica gel 300–400 meshand the eluents are indicated in parenthesis for each compound. The ^{13}C NMR spectral interpretation was done using numbering system indicated on the structure for curcumin (1) and the generalised structures in Tables 1 and 2.

3.1.1. General procedure for curcuminoid synthesis [22,23]

The aromatic or heterocyclic aromatic aldehyde (0.4 mol) and tributylborate [prepared by heating 2-butanol (44.4 g, 0.6 mol) and boric acid (12.4 g, 0.2 mol) in dry toluene using a Dean–Stark water separator] (210 mL, 184 g, 0.8 mol) were dissolved in dry ethyl acetate (200 mL). Acetylacetone–boric acid complex [prepared from acetyl acetone (20 g, 0.2 mol) and boric anhydride (10 g, 0.14 mol)] was added and after stirring the reaction mixture for 5 min, *n*-butylamine (4 mL) was added dropwise over a period 30 min. After stirring for 4 h the reaction mixture was left overnight at room temperature. Dilute hydrochloric acid (0.4 M, 300 mL) was added and the mixture was stirred in an oil bath at 60 °C for 1 h. After cooling the organic layer was separated and the aqueous layer was extracted several times with ethyl acetate or dichloromethane. The combined organic layer was washed with water, dried over anhydrous $MgSO_4$ and evaporated to yield a gummy product which crystallised from cold methanol. The crude curcuminoid was analysed by TLC [ethyl acetate:petroleum ether; 1:2] and further purified by flash chromatography using the same eluents.

The characteristic spectroscopic properties for all the synthesised curcuminoids RK78–RK107 were as follows:

1,7-Diphenyl-1,6-heptadiene-3,5-dione (*RK 78*), light-yellow powder; ^{1}H NMR: (δ, ppm, $CDCl_3$), 5.87 (1H, s, =CH_a), 6.65 (2H, d, $J = 15.5$ Hz, 2x CH_b=C), 7.27–7.42 (6H, m, 4x ArH_3 and 2x ArH_4), 7.50–7.60 (4H, m, 4x ArH_2), 7.68 (2H, d, $J = 15.5$ Hz, 2x C=CH_c); ^{13}C NMR: (δ, ppm,$CDCl_3$), 183.34 (C-3 and C-5), 140.65 (C-1 and C-7), 135.00 (C-1′), 130.14 (C-3′), 128.95 (C-2′), 128.14 (C-4′), 124.08 (C-2 and C-6), 101.82 (C-4); MS (EI, *m*/*z*), 288.1 (M^+, $C_{19}H_{16}O_2$, 100%), 131.1 (Ph–CH=CH–CO, 54%).

1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione (*RK 79*), orange powder; ^{1}H NMR: (δ, ppm, d^6-DMSO/$CDCl_3$), 3.47 (6H, s, 2x OCH_3), 5.41 (1H, s, 2x=CH_a), 6.03 (2H, d, $J = 15.0$ Hz, 2x CH_b=C), 6.42 (2H, d, $J = 8.3$ Hz, 2x ArH_3), 6.60 (2H, d, $J = 8.3$ Hz, 2x ArH_2), 6.65 (2H, s, 2x OH), 7.18 (2H, d, $J = 15.0$ Hz, 2x C=CH_c), 7.26 (1H, s, enolic OH); ^{13}C NMR: (δ, ppm, d^6-DMSO + $CDCl_3$), 183.32 (C-3 and C-5), 149.48 (C-3′), 148.21 (C-4′), 140.84 (C-1), 126.83 (C-1′), 123.00 (C-2), 121.18 (C-2′), 115.87 (C-3′), 110.62 (C-6′), 101.21 (C-4), 55.94 (OCH_3); MS (EI, *m*/*z*), 368.1 (M^+, $C_{21}H_{20}O_6$, 31%), 177.1 (Ar–CH=CH–CO, 100%).

1,7-Bis(4-methoxyphenyl)-1,6-heptadiene-3,5-dione (*RK 87*), orange powder; ^{1}H NMR: (δ, ppm, $CDCl_3$), 3.97 (6H, s, 2x OCH_3), 5.98 (1H, s,=CH_a), 6.65 (2H, d, $J = 15.2$ Hz, 2x CH_b=C), 6.92 (8H, d, 2x ArH AB system), 7.36 (2H, d, $J = 15.2$ Hz, 2x C=CH_c); ^{13}C NMR: (δ, ppm, d^6-DMSO + $CDCl_3$), 183.31 (C-3 and C-5), 161.33 (C-4′), 140.11 (C-1 and C-7), 129.88 (C-2′), 127.60 (C-1′), 121.81 (C-2 and C-6), 114.48 (C-3′), 101.47 (C-4), 55.47 (OCH_3); MS (EI, *m*/*z*), 336.1 (M^+, $C_{21}H_{20}O_4$, 62%), 161.0 (Ar–CH=CH–CO, 100%).

1,7-Bis(2-methoxyphenyl)-1,6-heptadiene-3,5-dione (*RK 88*), light orange powder; ^{1}H NMR: (δ, ppm, $CDCl_3$), 3.92 (6H, s, 2x OCH_3), 5.89 (1H, s,=CH_a), 6.74 (2H, d, $J = 16.0$ Hz, 2x CH_b=C), 6.92–7.01 (4H, m, 2x ArH_3 and 2x ArH_4), 7.32 (2H, t, $J = 7.8$ Hz, 2x ArH_5), 7.56 (2H, d, $J = 7.8$ Hz, 2x ArH_6), 8.00 (2H, d, $J = 16.0$ Hz, 2x C=CH_c); ^{13}C NMR: (δ, ppm, $CDCl_3$), 55.53 (OCH_3), 101.54 (C-4); 111.19 (C-3′), 120.76 (C-5′), 124.07 (C-2 and C-6), 124.80 (C-6′) 128.63

(C-4′), 131.30 (C-6′), 135.75 (C-1 and C-7), 158.40 (C-2′), 183.81 (C-3 and C-5); MS (EI, *m*/*z*), 336.2 (M^+, $C_{21}H_{20}O_4$, 67%), 161.1 (Ar–CH=CH–CO, 100%).

7-Bis(3,4-di-methoxyphenyl)-1,6-heptadiene-3,5-dione (*RK 89*), golden brown powder. ^{1}H NMR: (δ, ppm, $CDCl_3$), 3.92 (12H, m, 4x OCH_3), 5.82 (1H, s,=CH_a), 6.50 (2H, d, J = 15.5 Hz, 2x CH_b=C), 6.88 (2H, d, J = 8.2 Hz, 2x ArH_5), 7.08 (2H, s, 2x ArH_2), 7.14 (2H, d, J = 8.2 Hz, 2x ArH_6), 7.61 (2H, d, J = 15.5 Hz, 2x C=CH_c); ^{13}C NMR: (δ, ppm, $CDCl_3$),55.90 (OCH_3), 55.97 (OCH_3), 101.33 (C-4), 109.75 (C-2′), 111.12 (C-5′), 122.01 (C-2 and C-6), 122.63 (C-6′), 128.07 (C-1′), 140.38 (C-1 and C-7), 149.21 (C-4′), 151.17 (C-3′), 183.25 (C-3 and C-5); MS (EI, *m*/*z*), 396.2 (M^+, $C_{23}H_{24}O_6$, 1%), 191.0 (Ar–CH=CH–CO, 17%).

1,7-Bis(4-methylphenyl)-1,6-heptadiene-3,5-dione (*RK 90*), light mustard yellow powder. ^{1}H NMR: (δ, ppm, $CDCl_3$), 2.39 (6H, s, 2x CH_3), 5.83 (1H, s,=CH_a), 6.60 (2H, d, J = 16.0 Hz, 2x CH_b=C), 7.20 (4H, d, J = 7.8 Hz, AB system 2x ArH_3), 7.46 (4H, d, J = 7.8 Hz, AB system 2x ArH_2), 7.65 (2H, d, 16.0 Hz, 2x C=CH_c); ^{13}C NMR: (δ, ppm, $CDCl_3$), 183.40 (C-3 and C-5), 140.56 (C-1 and C-7), 140.52 (C-4′), 132.32 (C-1′), 129.68 (C-3′), 128.13 (C-2′), 123.12 (C-2 and C-6), 101.57 (C-4), 21.50 (CH_3); MS (EI, *m*/*z*), 304.3 (M^+, $C_{21}H_{20}O_2$, 46%), 145.1 (Ar–CH=CH–CO, 100%).

1,7-Bis(3-methoxyphenyl)-1,6-heptadiene-3,5-dione (*RK 91*), reddish orange powder. ^{1}H NMR: (δ, ppm, $CDCl_3$), 3.84 (6H, s, 2x OCH_3), 5.83 (1H, s,=CH_b), 6.59 (2H, d, J = 16.0 Hz, 2x CH_b=C), 7.39–6.89 (8H, m, ArH), 7.61 (2H, d, J = 16.0 Hz, C=CH_c); ^{13}C NMR: (δ, ppm, $CDCl_3$), 55.28 (OCH_3), 101.94 (C-4), 113.16 (C-2′), 115.95 (C-4′), 120.83 (C-6′), 124.35 (C-2 and C-6), 129.94 (C-5′), 136.43 (C-1′), 140.60 (C-1 and C-7), 159.93 (C-3′), 183.28 (C-3 and C-5); MS (EI, *m*/*z*), 336.3 (M^+, $C_{21}H_{20}O_4$, 39%), 161.1 (Ar–CH=CH–CO, 100%).

1,7-Bis(2-furanyl)-1,6-heptadiene-3,5-dione (*RK 92*), dark red oil. ^{1}H NMR: (δ, ppm, d^6-DMSO), 6.20 (1H, s,=CH_a), 6.57 (2H, d, J = 15.8 Hz, CH_b=C), 6.67 (2H, dd, J = 1.5 Hz and 3.1 Hz, 2x ArH_4), 6.97 (2H, d, J = 3.1 Hz, 2x ArH_5), 7.45 (2H, d, J = 15.8 Hz, 2x C=CH_c), 7.88 (2H, s, 2x ArH_3); ^{13}C NMR: (δ, ppm, $CDCl_3$), 183.30 (C-3 and C-5), 151.65 (C-2′), 143.75 (C-5′), 139.05 (C-1 and C-7), 129.40 (C-2 and C-6), 113.88 (C-3′), 112.82 (C-4′), 101.08 (C-4); MS (EI, *m*/*z*), 256.1 (M^+, $C_{15}H_{12}O_4$, 77%), 175 ($C_{10}H_7O_3$, 15%), 135 ($C_8H_8O_2$, 14%), 121 (Ar–CH=CH–CO, 100%), 107 (C_7H_7O, 25%), 94 (C_6H_6O, 31%), 81 (C_5H_5O, 41%) .

1,7-Bis(5-methylidenebutenolide)-1,6-heptadiene-3,5-dione (RK 97), sienna powder. ^{1}H NMR: (δ, ppm, $CDCl_3$), 3.99 (3H, s, OCH_3), 4.17 (3H, s, OCH_3), 5.80 (1H, s,=CH_a), 5.98 (2H, d, J = 11.3 Hz, 2x CH_d=C<), 6.18 (2H, d, J = 16.0 Hz, 2x CH_b=C), 7.27 (1H, s, OH), 7.61 (2H, dd, J = 11.3 Hz and 16.0 Hz, 2x C=CH_c); ^{13}C NMR: (δ, ppm, $CDCl_3$), 102.43 (C-5), 105.77 (C-1 and C-9), 125.85 (C-3′), 130.19 (C-3 and C-7), 131.36 (C-4′), 145.64 (C-5′), 148.17 (C-2 and C-8), 163.02 (C-2′), 182.45 (C-4 and C-6); MS (EI, *m*/*z*), 432 (M^+, $C_{21}H_{20}O_{10}$, 25%), 223 ($C_{11}H_{11}O_5$, 11%), 209 (Ar=CH–CH=CH–CO, 79%), 43 (100%).

1,7-Bis(2-thiophenyl)-1,6-heptadiene-3,5-dione (*RK 99*), reddish brown powder. ^{1}H NMR: (δ, ppm, $CDCl_3$), 5.75 (1H, s,=CH_a), 6.41 (2H, d, J = 15.5 Hz, 2x CH_b=C), 7.07 (2H, s, 2x ArH_3), 7.27 (2H, s, 2x ArH_4), 7.39 (2H, s, 2x ArH_5), 7.77 (2H, d, J = 15.5 Hz, 2x C=CH_c); ^{13}C NMR: (δ, ppm, $CDCl_3$), 182.85 (C-3 and C-5), 140.74 (C-2′), 133.36 (C-1 and C-7), 131.06 (C-5′), 128.39 (C-3′), 128.30 (C-4′), 123.04 (C-2 and C-6), 101.89 (C-4); MS (EI, *m*/*z*), 288.1 (M^+, $C_{15}H_{12}O_2S_2$, 49%), 270.1 (M-H_2O, 24%), 204.1 (M-C_4H_4S, 14%), 177 (M-C_6H_7S, 19%), 151 (C_8H_7OS, 32%), 137 (Ar–CH=CH–CO, 100%), 109 (C_6H_5S, 55%), 97 (C_5H_5S, 52%).

1,7-Bis(1-naphthyl)-1,6-heptadiene-3,5-dione (*RK 103*), golden yellow powder. ^{1}H NMR: (δ, ppm, $CDCl_3$), 5.97 (1H, s,=CH_a), 6.77 (2H, d, J = 15.0 Hz, 2x CH_b=C), 7.27 (1H, s, OH), 7.52–7.61 (6H, m, 2x ArH_3, ArH_6 and ArH_7), 7.82–7.90 (6H, m, 2x ArH_4, ArH_5 and ArH_8), 8.28 (2H, d, J = 7.7 Hz, 2x ArH_2), 8.56 (2H, d, J = 15 Hz, 2x C=CH_c); ^{13}C NMR: (δ, ppm, $CDCl_3$), 102.26 (C-4), 123.49 (C-2′), 124.93 (C-2 and C-6), 125.51 (C-8′), 126.28 (C-6′), 126.56 (C-7′), 126.91 (C-3′), 128.78 (C-5′), 130.50 (C-4′), 131.60 (C-9′), 132.37 (C-10′), 133.77 (C-1′), 137.53 (C-1 and C-7), 183.32 (C-3 and C-5); MS (EI, *m*/*z*), 376.3 (M^+, $C_{27}H_{20}O_2$, 3%), 195 ($C_{14}H_{12}O$, 2%), 181 (Ar–CH=CH–CO, 3%).

1,7-Bis[2-(5-methylthiophenyl)]-1,6-heptadiene-3,5-dione (*RK 104*), firebrick powder. ^{1}H NMR: (δ, ppm, $CDCl_3$), 2.51 (6H, s, 2x CH_3), 5.70 (1H, s,=CH_a), 6.27 (2H, d, J = 15 Hz, 2x CH_b=C), 6.73 (2H, d, J = 3.6 Hz, 2x ArH_4), 7.06 (2H, d, J = 3.6 Hz, 2x ArH_3), 7.68 (2H, d, J = 15 Hz, 2x C=CH_c); ^{13}C NMR: (δ, ppm, $CDCl_3$), 15.92 (CH_3), 101.56 (C-4), 121.77 (C-2 and C-6), 126.76 (C-4′), 131.64 (C-1 and C-7), 133.42 (C-3′), 138.66 (C-2′), 144.09 (C-5′), 182.74 (C-3 and C-5); MS (EI, *m*/*z*), 316.3 (M^+, $C_{17}H_{16}O_2S_2$, 42%), 298.3 (M-H_2O, 31%), 218.3 ($C_{12}H_{10}O_2S$, 17%), 191.2 ($C_{10}H_7O_2S$, 15%), 165 (C_9H_9OS, 32%), 151.2 (Ar–CH=CH–CO, 86%), 111.1 (C_6H_7S, 40%), 45 (100%).

1,7-Bis[2-(3-methylthiophenyl)]-1,6-heptadiene-3,5-dione (*RK 105*), reddish brown powder. ^{1}H NMR: (δ, ppm, $CDCl_3$), 2.36 (6H, s, 2x CH_3), 5.70 (1H, s,=CH_a), 6.34 (2H, d, J = 15.5 Hz, 2x CH_b=C), 6.87 (2H, d, J = 5.1 Hz, 2x ArH_4), 7.26 (2H, d, J = 5.1 Hz, 2x ArH_5), 7.83 (2H, d, J = 15.5 Hz, 2x C=CH_c); ^{13}C NMR: (δ, ppm, $CDCl_3$), 14.24 (CH_3), 102.06 (C-4), 122.36 (C-2 and C-6), 127.10 (C-2′), 131.47 (C-4′), 131.58 (C-5′), 133.15 (C-1 and C-7), 141.45 (C-3′), 182.80 (C-3 and C-5); MS (EI, *m*/*z*), 316 (M^+, $C_{17}H_{16}O_2S_2$, 53%), 165 (C_9H_9OS, 16%), 151.0 (Ar–CH=CH–CO, 100%), 111 (C_6H_7S, 59%).

1,7-Bis[2-(5-methyfuranyl)]-1,6-heptadiene-3,5-dione (*RK 106*), maroon powder. ^{1}H NMR: (δ, ppm, $CDCl_3$), 2.38 (6H,s, 2x CH_3), 5.71 (1H, s,=CH_a), 6.09 (2H, d, J = 3.6 Hz, 2x ArH_4), 6.42 (2H, d, J = 15.5 Hz, 2x CH_b=C), 6.51 (2H, d, J = 3.1 Hz, 2x ArH_3), 7.33 (2H, d, J = 15.5 Hz, 2x C=CH_c); ^{13}C NMR: (δ, ppm, $CDCl_3$), 13.97 (CH_3), 102.08 (C-4), 109.19 (C-4′), 116.72 (C-3′), 120.15 (C-2 and C-6), 126.75 (C-1 and C-7), 150.39 (C-2′), 155.55 (C-5′), 182.84 (C-3 and C-5); MS (EI, *m*/*z*), 284.4 (M^+, $C_{17}H_{16}O_4$, 33%), 266.2 (M-H_2O, 6%), 149 ($C_9H_{10}O_2$, 8%), 135 (Ar–CH=CH–CO, 67%), 108.2 (C_7H_8O, 12%), 95.2 (C_6H_7O, 32%), 45 (100%).

1,7-Bis(3,4-methylenedioxyphenyl)-1,6-heptadiene-3,5-dione (*RK 107*), golden yellow powder. ^{1}H NMR: (δ, ppm, d^6-DMSO/$CDCl_3$), 5.73 (5H, s, 2x –OCH_2– and =CH_a), 6.18 (2H, d, J = 15.8 Hz, 2x CH_b=C), 6.53 (2H, d, J = 8.1 Hz, 2x ArH_5), 6.74 (2H, d, J = 8.1 Hz, 2x ArH_6), 6.76 (2H, d, J = 15.8 Hz, 2x C=CH_c), 7.27 (2H, s, 2x ArH_2); ^{13}C NMR: (δ, ppm, d^6-DMSO), 183.62 (C-3 and C-5), 149.76 (C-3′), 148.62 (C-4′),140.61 (C-1 and C-7), 129.67 (C-1′), 125.52 (C-2 and C-6), 122.81 (C-6′), 109.09 (C-5′), 107.00 (C-2′), 102.10 (O–CH_2–O), 101.94 (C-4); MS (EI, *m*/*z*), 364 (M^+, $C_{21}H_{16}O_6$, 5%), 232 ($C_8H_4O_2$, 33%), 189 ($C_{11}H_9O_3$, 19%), 175 (Ar–CH=CH–CO, 37%), 149 ($C_9H_9O_2$, 62%), 99 (73%), 57 (100%).

3.2. Pharmacology

3.2.1. In vivo and in vitro activities

Anti-inflammatory activity (AIA) was assessed after oral feeding in female Wistar rats by two methods:

(A) Acute AIA was determined using the standard carrageenan-induced paw oedema assay [30,31]. Female rats were employed as these express greater inflammation in paws and joints than in male animals of the same strain. Moreover, it is well-recognised that rheumatoid arthritis (of which AIA attempts to replicate) is expressed predominantly in females. Changes in paw thickness were measured with a micrometer 1, 2, and 24 h after inoculating Na carrageenan (0.6 mg in 0.1 ml physiological saline) into each rear paw. Test agents in reference drugs were administered orally suspended in 0.02% Tween-20 and 5% (v/v) ethanol 45 min before inoculating carrageenan.

(B) Chronic AIA was determined by suppression of arthritis in rats developing adjuvant-induced polyarthritis [31,32]. Test agents

and reference drugs were administered once daily for four days only beginning on day-10; day-0 = day of inoculating adjuvant into tail-base. In some rats the prostaglandin analogue, misoprostol, was co-administered with curcuminoid in order to enhance the expression of anti-inflammatory activity of the latter [32]. The following signs of arthritis were measured on days-10, 14 and 17 (the latter measurement indicated rebound after ceasing dosing on day-13): (a) rear paw thickness, (b) fore paw inflammation, (c) an overall arthritis score (these last two measurements made subjectively on a scale 0 → 4+), and (d) weight changes over days 10 through to 14.

Gastro-irritancy was measured in both normal and arthritic rats fasted overnight, dosed orally with test compounds (±0.13 M HCl in water), then sacrificed 2.5 h later [34]. The stomachs were excised, lightly rinsed with water and point haemorrhagic lesions enumerated, and the gastric lesion index was then calculated [33,34].

Anti-pyretic activity was measured in yeast-fevered rats [33].

3.2.2. Ethical approval

The animal studies were conducted under protocols approved by the University of Queensland Animal Ethics Committee-5.

3.2.3. In vitro production of PGE2 and cytokine inflammatory mediators

Since previous reports have shown that curcumin may affect production of prostaglandins and pro-inflammatory cytokines [35,36] it was decided to measure production of the mediators in response to inflammatory stimuli in THP-1 monocytes *in vitro*. The procedures employed were those as described by Rainsford et al. [37]. The production of prostaglandinE_2 (PGE_2), interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) from THP-1 monocyte cells in the presence and absence of *Escherichia coli* lipopolysaccharide (LPS 100 μg/mL; Sigma). The cells were initially cultured in Dulbecco's Modified Eagle's Medium (DMEM) with 5% heat inactivated foetal calf serum (FCS). Prior to incubation with the drugs and/or LPS, the cells were centrifuged and re-suspended in DMEM without FCS and 10^6 cells in 0.5 mL were plated out in 24-well sterile culture dishes. Some of the compounds proved difficult to dissolve in 0.2% DMSO alone (as employed with NSAIDs previously [37], so they were prepared in 100 μM final concentrations dissolved in 0.2% DMSO with 0.6% bovine serum albumin added to assist their uptake into the cells. To stimulate production of prostaglandins and cytokines LPS 100 μg/mL in DMSO was added and the cells then incubated for 24 h. The media was collected and assayed for PGE_2, IL-1-β or TNF-α using the manufacturer's (R&D Systems) guidelines [37].

3.2.4. Production of PGE_2 and cytokines in plasma of arthritic rats

A limited number of plasma samples were collected from the arthritic rats treated with curcuminoids (as above), stored and shipped in dry ice for subsequent assay of PGE_2, IL-1-β or TNF-α, which was performed without dilutions using the manufacturer's (R&D Systems) guidelines [37].

4. Discussion and conclusion

The results show that there is variability on the acute versus chronic anti-inflammatory activities *in vivo* as well as their *in vitro* effects on production of key inflammatory mediators that are important in manifestations of arthritic and other painful conditions. Of the curcuminoids investigated in these studies RK-92, RK-97, RK-103, RK-104, RK-106 and RK-107 inhibited inflammatory reactions *in vivo*. Yet paradoxically, the furan RK-106 was the only curcuminoid that affected production of the pro-inflammatory cytokines, IL-1β and TNFα. The inhibition of TNFα is a most significant effect since it is the target of modern anti-cytokine biologics [38], but have disadvantages because of some risks of stimulating infections (e.g. tuberculosis, hepatitis) [39]. The anti-inflammatory effects of RK-106 may represent a lead for the development of prototype curcuminoids with anti-TNFα activity for the therapy of chronic rheumatic diseases.

The negative results with curcumin itself in the acute anti-inflammatory and anti-pyretic assays suggest that any ability of curcuminoids to inhibit cyclooxygenase/lipoxygenase or proinflammatory cytokines systems *in vitro* [35,36], may not be rapidly expressed, if at all, *in vivo*. This may reflect slow absorption, rapid detoxification, binding to endogenous ligands for beta-diketones e.g. thiol groups or yet other factors reducing/slowing bioavailability [40,41].

Acknowledgments

These studies were supported by the Anti-inflammatory Research Fund of Professor K D Rainsford and the Biomedical Research Centre, Sheffield Hallam University. We are grateful to Professor M.S. Roberts (Department of Medicine, University of Queensland) for providing animal facilities. Our thanks to Mr. Alexander Rainsford for his expert preparation of the figures.

References

[1] B.B. Aggarwal, Y.J. Surh, S. Shishodia (Eds.), The Molecular Targets and Therapeutic Uses of Curcumin in Health and Disease, Springer, New York, 2007.
[2] S. Mills, K. Bone, Principles and Practice of Phytotherapy, Churchill Livingstone, Edinburgh, 2000.
[3] L. Braun, N. Cohen, Herbs and Natural Supplements: An Evidence-Based Guide, Elsevier-Mosby, Sydney, 2004. pp. 358–368.
[4] R.C. Srimal, B.N. Dhawan, J. Pharm. Pharmacol. 5 (6) (1973) 447–452.
[5] A.N. Nurfina, M.S. Reksohadiprodja, H. Timmerman, U.A. Jenie, D. Sugiyaanto, H. van der Goot, Eur. J. Med. Chem. 32 (4) (1997) 321–3285.
[6] A. Mukhopadhyay, N. Basu, N. Ghatak, P.K. Gujral, Agents Actions 12 (4) (1982) 508–515.
[7] J.K. Jackson, T. Higo, W.L. Hunter, H.M. Burt, Inflamm. Res. 55 (4) (2006) 168–175.
[8] P. Anand, C. Sundaram, S. Jhurani, A.B. Kunnumakkara, B.B. Aggarwal, Cancer Lett. 267 (1) (2008) 133–164.
[9] I. Brouet, H. Ohshimina, Biochem. Biophys. Res. Commun. 206 (2) (1995) 533–540.
[10] T. Osawa, Y. Sugiyama, M. Inayoshi, S. Kawakishi, Biosci. Biotech. Biochem. 59 (9) (1995) 1609–1612.
[11] H. Ahsan, N. Parveen, N.U. Khan, S.M. Hadi, Chem.–Biol. Interact. 121 (2) (1999) 161–175.
[12] M.H. Pan, S.Y. Lin-Shiau, J.K. Lin, Biochem. Pharmacol. 60 (11) (2000) 1665–1676.
[13] J. Hong, M. Bose, J. Ju, J.H. Ryu, X. Chen, S. Sang, M.J. Lee, C.S. Yang, Carcinogenesis 25 (2004) 1671–1679.
[14] S. Bengmark, J. Parent Enter. Nutrit. 30 (1) (2006) 45–51.
[15] I. Rahman, S.K. Biswas, P.A. Kirkham, Biochem. Pharmaco. 72 (11) (2006) 1437–1452.
[16] S.K. Sandur, M.K. Pandey, B. Sung, K.S. Ahn, A. Murakami, G. Sethi, P. Limtrakul, V. Badmaev, B.B. Aggarwal, Carcinogenesis 28 (8) (2007) 1765–1773.
[17] Y. Henrotin, A.L. Clutterbuck, D. Allaway, E.M. Lodwig, P. Harris, M. Mathy-Hartert, M. Shakibaei, Osteoarth. Cartilage 18 (2010) 141–149.
[18] S. Lev-Ari, L. Strier, D. Kazanov, O. Elkayam, D. Lichtenberg, D. Caspi, N. Arber, Rheumatology 45 (2) (2006) 171–177.
[19] H. Itokawa, Q. Shi, T. Akiyama, S. Morris-Natschke, K.H. Lee, J. Nat. Med. (2008) 263–280.
[20] J.R. Anto, G. Kuttan, K.V.D. Babu, K.N. Rajasekharan, R. Kuttan, Pharm. Pharmcol. Commun. 4 (1997) 103–106.
[21] V. Lampe, J. Milobedska, St. V. Kostanecki, Ber. 43 (1910) 2163.
[22] H.J.J. Pabon, Rec. Trav. Chim. Pays – Bas. 83 (1964) 379–386.
[23] U. Pedersen, P.B. Rasmussen, S.O. Lawesson, Liebigs Ann. Chem. (1985) 1557–1569.
[24] P.J. Roughley, D.A. Whiting, J. Chem. Soc. Perkin 1 20 (1973) 2379–2387.
[25] R. El-Khatib, The Synthesis of Curcumins and Other Related Compounds (Work Placement Report), Sheffield Hallam University, 2000 (October).
[26] X. Qiu, Z. Liu, W.-Y. Shao, X. Liu, D.-P. Jing, Y.-J. Yu, L.-K. An, S.-L. Huang, X.-Z. Bu, Z.S. Huang, L.-Q. Gu, Bioorgan. Med. Chem. 16 (2008) 8035–8041.
[27] M.A. Khan, H. Adams, Synthesis 1 (1995) 687–692 (Thieme).
[28] M.A. Khan, S.A. Boyes, H. Adams, Molecules 1 (1996) 27–36.
[29] P. Anand, A. Kunnumakkara, R. Newman, B.B. Aggarwal, Mol. Pharmacol. 4 (2007) 807–818.

[30] C.A. Winter, E.A. Risley, G.W. Nuss, Proc. Soc. Exp. Biol. Med. 111 (1962) 544–547.
[31] J.M. Snowden, M.W. Whitehouse, Inflammopharmacology 5 (1997) 127–132.
[32] M.W. Whitehouse, B. Vernon-Roberts, Inflammopharmacology 1 (1991) 61–68.
[33] K.D. Rainsford, M.W. Whitehouse, Agents Actions 10 (1980) 451–456.
[34] K.D. Rainsford, M.W. Whitehouse, Inflammopharmacology 14 (2006) 150–154.
[35] M.T. Huang, T. Lysz, T. Ferrero, et al., Cancer Res. 51 (1991) 813.
[36] S.M. Plummer, K.A. Holloway, M.M. Manson, et al., Oncogene 18 (1999) 6013.
[37] K.D. Rainsford, H. Omar, A. Ashra, A.T. Hewson, R.A.D. Bunning, R. Rishiraj, P. Shepherd, R.W. Seabrook, Inflammopharmacology 10 (2002) 185–239.
[38] M. Feldmann, S.R. Maini, Immunol. Rev. 223 (2008) 7–19.
[39] J.B. Galloway, K.L. Hyrich, L.K. Mercer, W.G. Dixon, B. Fu, A.P. Ustianowski, K.D. Watson, M. Lunt, D.P. Symmons, Rheumatology (Oxford) (2011) 50 124–131.
[40] M.W. Whitehouse, Arthritis Rheum. 12 (1969) 342.
[41] D.E. Butters, M.W. Whitehouse, Inflammopharmacology 11 (2003) 97–110.