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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological evaluation of novel curcumin analogues as anti-inflammatory, anti-cancer and anti-oxidant agents

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Abstract A series of novel curcumin analogues **5a–m** were synthesized by Claisen-Schmidt condensation of various aromatic and heteroaromatic amides of 3-aminoactophenones **4a–m** with 3-bromo-2,4,6-trimethoxybenzaldehyde and characterized by IR, ^{1}H NMR and mass spectroscopic analysis and were evaluated for anti-inflammatory, anti-cancer and anti-oxidant activity. Out of the 13 synthesized compounds, compounds **5f**, **5j** and **5m** were excellent inhibitors of TNF-α and IL-6. Compounds **5c**, **5e**, **5b** and **5d** showed potent COX-2 inhibition, compounds **5d** and **5f** have shown good trypsin inhibition and compounds **5e**, **5g** and **5c** exhibited excellent β-glucuronidase inhibition. Compounds **5l** and **5m** showed potent anti-cancer activity against selected five human cancer cell lines. All the compounds exhibited moderate free radical scavenging activity, while compounds **5a** and **5m** were excellent OH radical scavengers.

Keywords TNF-α · IL-6 · COX-2 · Anti-cancer activity · Anti-oxidant activity

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Introduction

Curcumin (diferuloylmethane) (Fig. 1) is a β-diketone constitute of the turmeric obtained from the powdered root of *Curcuma longa* Linn. This yellow pigment is the main constituent of turmeric powder, a widely used spice in Southeast Asia (Nadkarni, 1976). Curcumin is also used in traditional medicine to treat wide variety of human ailments such as indigestion, urinary tract infection and liver diseases (Cooper *et al.*, 1994). Curcumin and its analogues are also reported to possess anti-inflammatory, anti-oxidant, anti-proliferative, anti-angiogenic and anti-tumorigenic properties (Ammon and Wahl, 1991; Kawamori *et al.*, 1999; Huang *et al.*, 1997, 1988).

Amongst the series of newly invented drug targets for inflammations and related disorders, tumour neurosis factor-α (TNF-α) has been described as a pro-inflammatory cytokine produced by monocytes, macrophages, neutrophils, T-cells, epithetial cells, osteoblasts and dendritic cells (Mehta *et al.*,1997; Tracy and Cerami, 1994). The overexpression of TNF-α is responsible for number of pathological disorders like ulcerative colitis (Goldfeld *et al.*, 1991), diabetes (Newton and Decicco, 1999), multiple sclerosis (Hotamisligil *et al.*, 1995), atherosclerosis (Selmaj *et al.*, 1991) and stroke (Rus *et al.*, 1991). Moreover, there exist an evidence to support the fact that TNF-α plays a key role in the origin and progression of rheumatoid arthritis (RA) and other immune related disorders (Lovering and Zhang, 2005; Oppenheim and Feldmann, 2001; Feldmann *et al.*, 1996; Odeh, 1997; Bertolini *et al.*, 1986; Sakalatvala, 1986; Papadakis and Targan, 2000; Tracey *et al.*, 2008). After the role of TNF-α was elucidated clearly in RA, efforts have been made to develop inhibitors of TNF-α. Its activity may be potentially inhibited at a variety of sites. There are several strategies to inhibit the

production or release of TNF-α from the cell, neutralize TNF-α on the cell surface or in the soluble phase or block the TNF-α receptor or its downstream signal transduction pathway (Elliott and Maini, 1994; Moreland *et al.*, 1997).

Amongst pro-inflammatory cytokines, interleukin-6 (IL-6) is considered to play a key role of initiation and extension of the inflammatory process. IL-6 is a multifunctional cytokine produced by wide range of cells, usually at site of tissue injury, and it regulated hepatic acute phase response, the immune response, inflammation and haematopoiesis. It appears to be the central mediator in a range of inflammatory diseases, including end-stage renal disease and rheumatoid arthritis (Dominic, 2009). Inhibition of IL-6 has received desired attention in drug discovery.

Result and discussion

Although, curcumin is non-toxic and has promising biological activities, preclinical and clinical studies have indicated that poor bioavailability and pharmacokinetic profile are due to its instability under physiological condition which has limited its application in anti-cancer therapies (Hsu and Cheng, 2007; Pan *et al.*, 1999; Sharma *et al.*, 2007). Evidences from both in vitro and in vivo studies show that β-diketone moiety is responsible for instability and weak pharmacokinetic profiles of curcumin. During last decade synthetic modifications of curcumin, which were aimed at enhancing its bioactivities, suggested that the stability and metabolic profile of curcumin could be enhanced by deleting β-diketone moiety. To overcome these barriers, several research groups have synthesized curcumin analogues. Robinson *et al.* (2005) have synthesized enone and dienone analogues, Ahn *et al.* (2004) have synthesized the bis-alkynyl/alkyl, pyridine and thiophene derivatives as curcumin analogues. Woo *et al.* (2005) have synthesized curcumin mimics containing enone and amide ring (Fig. 1). Herein, we have synthesized the novel curcumin analogues containing enone and amide containing trimethoxy benzene moiety.

The 1,3,5-trimethoxybenzene on Vilsmeier-Haack formylation gives 2,4,6-trimethoxy benzaldehyde (**2**), which on bromination with bromine in glacial acetic acid afford 3-bromo-2,4,6-trimethoxybenzaldehyde (**3**). Compounds (**4a**–**m**) were prepared by acylation of 3-aminoacetophenone with different acylchlorides in basic medium. Compounds (**4a**–**m**) on Claisen-Schmidt condensation with 3-bromo-2,4,6-trimethoxybenzaldehyde (**3**) under basic media afforded a residue, which on purification by column chromatography with 1% ammonia and 0.5–1% methanol in chloroform as eluting solvent, furnished title compounds (**5a**–**m**) in good yields (Scheme 1). All the synthesized curcumin analogues were characterized by IR, ^{1}H NMR and mass spectral analysis.

All synthesized compounds were evaluated for anti-inflammatory (TNF-α, IL-6, COX-1 and -2, β-glucuronidase and trypsin), anti-cancer and antioxidant activity. Anti-inflammatory activity against TNF-α and IL-6 and results are presented in Table 1. Results revealed that the synthesized compounds **5a**, **5j**, **5b**, **5c**, **5f**, **5h**, **5g** and **5m** have shown excellent inhibiton of TNF-α (91–83%) as compared with standard dexamethasone (72%). Overall, compounds **5f**, **5j** and **5m** are the most active among the series against TNF-α and have least cytotoxicity against CCK-8 cell line. The structure activity relationship study in these compounds revealed that compounds containing electron-withdrawing substituent on amide ring have higher TNF-α inhibition than their counterpart containing electron-donating substituent on amide ring with exception compound **5g**. Bioisosteric replacement of aromatic ring by thiophene there is slight decrease in TNF-α inhibition.

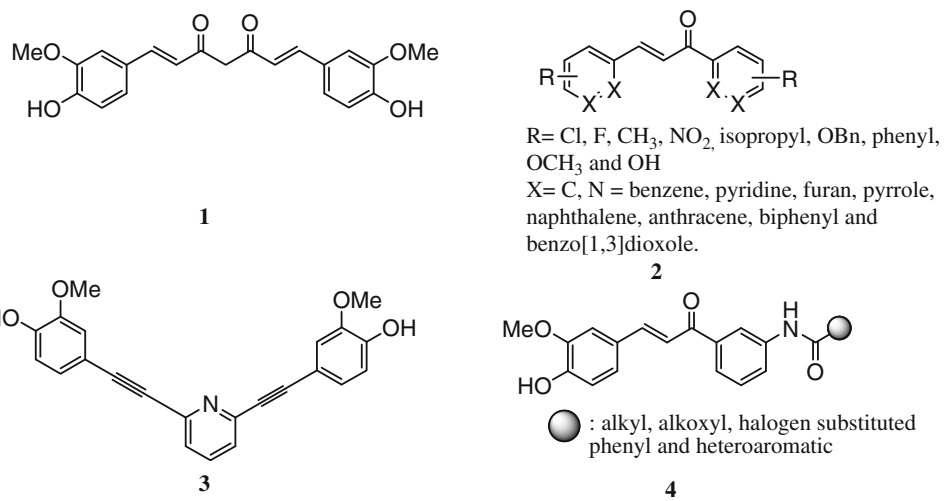
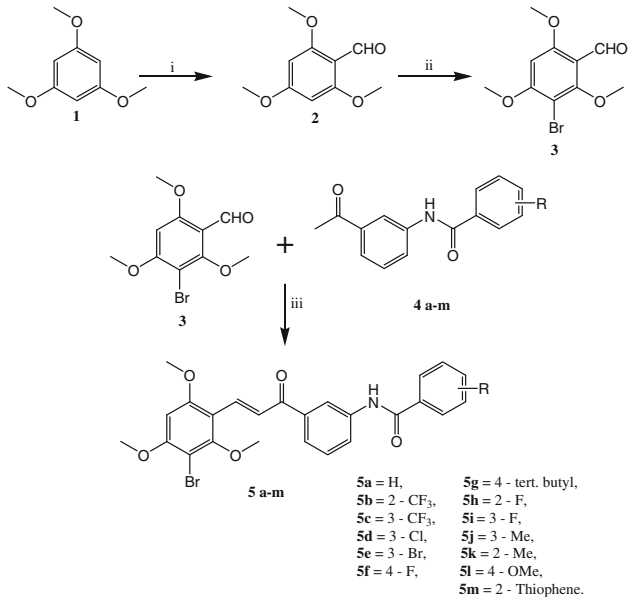


Fig. 1 Curcumin and its analogues



Scheme 1 Synthesis of curcumin analogues

Table 1 Anti-inflammatory activity against TNF-α and IL-6

Compounds	% Inhibition at 10 μM		
	TNF-α	IL-6	Toxicity
5a	91	98	65
5b	85	95	62
5c	85	97	65
5d	00	64	25
5e	04	75	31
5f	85	97	49
5g	83	96	56
5h	84	96	56
5i	78	93	51
5j	87	97	56
5k	72	95	54
5l	63	94	65
5m	80	96	53
DMS (1 μM)	72	94	00

DMS Dexamethasone, The results summarized are the mean values of $n = 2$

The synthesized compounds screened for IL-6 inhibition and results are summarized in Table 1. Results showed that synthesized compounds are found to be active against IL-6. Compounds **5a**, **5c**, **5f**, **5j**, **5g**, **5h**, **5m** and **5b** have shown excellent inhibition of IL-6 (98–95%) as compared with standard dexamethasone (94%). Overall, compounds **5f**, **5j** and **5m** are most active and have lowest cytotoxicity against CCK-8 cell line. Structure activity relationship indicates amides containing electron-withdrawing substituents are essential for IL-6 inhibition, with exception

Table 2 Anti-inflammatory activity of novel curcumin analogues at 1 μM conc

Compounds	COX-1 (%)	COX-2 (%)	Trypsin (%)	β-glucuronidase (%)
5a	51.91	65.67	58.12	49.61
5b	39.86	87.88	68.41	76.86
5c	48.89	89.25	65.48	81.38
5d	53.87	86.51	78.11	78.24
5e	41.71	88.38	66.45	84.68
5f	58.92	83.28	72.86	79.39
5g	64.56	85.79	69.58	82.54
5h	44.78	79.31	64.75	74.24
5i	59.76	82.54	63.54	72.89
5j	24.18	48.22	51.61	66.91
5k	31.49	43.71	35.52	29.72
5l	19.55	37.95	39.63	33.98
5m	27.81	55.41	41.37	44.67
ASA	–	36.93	–	–
SC 560	36.71	–	–	–
SA	–	–	84.99	24.77

The results summarized are the mean values of $n = 2$

ASA acetyl salicylic acid, *SA* salicylic acid, *SC560* a standard COX-1

compound **5g**. Compounds with fluorine and trifluoromethyl substitution have highest activity than the compounds containing chloro and bromo substitution on amide ring.

The synthesized compounds are subjected to COX inhibition study and results are shown in Table 2. Compounds **5c**, **5e**, **5b**, **5d** and **5f** have shown potent (89.25–83.28%) COX-2 inhibition. COX-2 is expressed during inflammation and COX-1 is constitutive, which is involved in physiological functions. Therefore, compounds selectively inhibit the COX-2 have good account of anti-inflammatory activity. Compounds under investigation have inhibited COX-2 preferentially and are more selective towards COX-2 rather than COX-1. Structure–activity relationship study with respect to cyclooxygenase inhibition revealed that compounds containing electron-withdrawing substituent on amide ring with exception **5g**. Change in the position of substituent will affect the activity, changing the position of fluorine from para (**5f**) to meta (**5i**) there is a slight decrease in activity and from meta (**5i**) to ortho (**5h**) further decrease in activity.

Trypsin is a member of the serine proteases family. These proteases are involved in initiation of inflammation; moreover, serine protease inhibition has been considered as one of the target for design of anti-inflammatory drugs (Bilfinger and George, 2002). From Table 2, compounds **5d** (78.11%) and **5f** (72.86%) have shown good trypsin inhibition, while compounds **5g**, **5b**, **5e**, **5c** and **5h** have shown moderate trypsin inhibiton (69.58–64.75%) as

compared with standard salicylic acid (84.99%). Electron-withdrawing groups on amide ring are essential for trypsin inhibition with exception compound **5g**.

The enzyme β-glucuronidase has been considered as one of the targets in the design of anti-inflammatory agents as it play the role in the initiation of inflammation. The lysosomes of the polymorphonuclear neutrophils are rich in β-glucuronidase. This enzyme is attributed as one of the mediators for initiating the process of inflammation. Compounds **5e**, **5g**, **5c**, **5f**, **5b** and **5h** have shown excellent inhibition of β-glucuronidase (84.68–74.24%) as compared with standard salicylic acid (24.77%). Compounds with electron-withdrawing groups on amide ring are essential for the β-glucuronidase inhibition than the compounds containing electron-donating groups. The aromatic amide (**5a**) and heteroaromatic amide (**5m**) are weak inhibitors of β-glucuronidase.

All the synthesized compounds were screened for anti-cancer activity against ACHN (renal cell carcinoma), Panc1 (pancreatic carcinoma), Calu1 (non small cell lung carcinoma), H460 (non small cell lung carcinoma) and HCT116 (colon carcinoma) human cancer cell lines (Table 3). Compounds **5l** and **5m** have shown potent anti-cancer activity against selected five cancer cell lines as compared with standard flavopiridol and gemcitabine. Compound **5h** has good anti-cancer activity against all five cancer cell lines. Structure–activity relationship study revealed that compounds containing electron-donating groups on heteroaromatic amide ring have the highest cytotoxicity. These compounds showing a broad-spectrum inhibition against all selected five human cancer cell lines.

Table 3 Anti-cancer activity of curcumin analogues at 10 μM conc

Compounds	Panel	H460	Calu-1	hct116	ACHN
5a	10	14	06	00	21
5b	03	11	00	06	06
5c	00	04	00	06	15
5d	35	17	26	28	26
5e	00	06	00	00	06
5f	66	62	60	64	69
5g	37	51	32	10	35
5h	68	52	67	66	66
5i	32	33	31	26	33
5j	23	32	22	30	28
5k	03	32	00	21	15
5l	99	98	99	98	97
5m	89	72	85	98	82
Flavopiridol (700 nM)	68	73	82	83	73
Gemcitabine (500 nM)	78	79	87	86	84

$Panc_1$ human pancreas carcinoa, *H_{460}* humann non cell lung carcinoma, *Calu-1* human non cell lung carcinoma, *hct 116* human colon carcinoma, *ACHN* human renal cell carcinoma

Table 4 Summary of the results of DPPH, OH radical scavenging activity (%) and reducing activity (%) of curcumin analogues at 1 μM conc

Compounds	DPPH (%)	OH (%)	Reducing activity (%)
5a	49.44	54.14	46.62
5b	44.65	35.85	42.87
5c	45.69	NR	40.69
5d	45.34	NR	38.41
5e	42.56	23.65	39.68
5f	44.30	33.41	43.92
5g	43.61	27.31	36.81
5h	42.91	43.17	40.55
5i	43.26	32.19	39.85
5j	36.66	38.29	29.22
5k	31.11	34.63	22.31
5l	36.31	21.21	26.94
5m	35.27	49.26	32.66
GA (1 μM)	92.36	–	–
AA(1 mM)	–	45.85	78.89

GA gallic acid, *AA* ascorbic acid, *NR* no reaction

According to recent studies, free radicals such as reactive oxygen species (ROS) are important mediators that initiate and propagate inflammatory responses by stimulating release of pro-inflammatory cytokines such as IL-1β and TNF-α (Geronikaki and Gavalas, 2006). It was also found that ROS induced by activated neutrophils, eosinophils, monocytes and macrophages during the inflammation process leads to tissue injury by damaging macromolecules and affecting the lipid peroxidation of membranes (Gutteridge, 1995). The anti-oxidant therapy is coming up as an attractive therapeutic approach for variety of human ailments. The results of anti-oxidant activity of novel curcumin analogues are summarized in Table 4. Compounds **5a**, **5c**, **5d**, **5e** and **5f** exhibited moderate free radical scavenging activity (49.44–44.30%) as compared with standard gallic acid (92.30%).

Compounds **5a** (54.14%) and **5m** (49.26%) have shown potent hydroxyl radical scavenging ability as compared with ascorbic acid (45.85%), while compounds **5h**, **5j**, **5b** and **5k** have shown good hydroxyl radical scavenging activity. Aromatic amide (**5a**) and heteroaromatic nucleus is required for hydroxyl radical scavenging activity. Compounds **5a**, **5f**, **5b**, **5c**, **5h** and **5e** showed moderate reducing activity.

Experimental

General methods

Chemicals were purchased from Aldrich Chemical Co., USA. Melting points were determined with a digital

thermometer were uncorrected. IR spectra were recorded on FT-IR Shimadzu 8300 spectrophotometer and ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrometer in $CDCl_3$ using tetramethylsilane as an internal standard and chemical shifts are reported in δ units and the coupling constants (J) are reported in hertz. Mass spectra were obtained with a Shimadzu LCMS-2010 EV. Chromatographic purification was performed with silica gel (100–200 mesh). Thin layer chromatography was performed on pre-coated silica plates (Merck Kiesegel $60F_{254}$, 0.2 mm thickness) sheets. The spots could be visualized easily under ultraviolet light.

*General procedure for the synthesis of curcumin analogues (**5a–m**)*

Amides **5a**–**m** (1 mmol) were dissolved in ethanol (15 ml), NaOH (20%) was added to it and stirred for 5 min. 3-bromo-2,4,6-trimethoxybenzaldehyde **3** (1 mmol) was added and stirring continued for 24 h at room temperature. After completion of reaction (TLC), reaction mixture was poured over crushed ice, extracted with chloroform, organic extract was washed with water, dried (anhydrous Na_2SO_4) and concentrated. Purification was carried using silica gel column using mixture of 0.5–1% methanol + 1% liquor ammonia in chloroform as an eluent to obtain the title compounds (**5a**–**m**).

*(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl)acryloyl) phenyl)benzamide (**5a**)* (yield 58%); mp 141–143°C; IR υ_{max} cm^{-1} (KBr): 3470, 3168, 2989, 2857, 1618, 1614, 1597, 1567, 1540, 1510, 1410, 1017, 945, 844, 763; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.13 (1H, d, $J = 2.4$ Hz), 8.10 (1H, m), 7.92 (2H, d, $J = 8.2$ Hz), 7.52 (4H, m, Ar–H), 7.26 (1H, s, –NH), 6.35 (1H, s, Ar–H), 3.96 (6H, s, $-OCH_3 \times 2$), 3.84 (3H, s, $-OCH_3$). MS: m/e 497 (M + 1). *Anal.* Calcd. For $C_{25}H_{22}BrNO_5$: C 60.50, H 4.47, Br 16.10, N 2.82, O 16.12. Found: C 60.53, H 4.50, Br 16.14, N 2.84, O 16.14.

*N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl)acryloyl) phenyl)-2-trifluorobenzamide (**5b**)* (yield 60%); mp 182–184°C; IR υ_{max} cm^{-1} (KBr): 3417, 3145, 2968, 2840, 1618, 1608, 1598, 1540, 1510, 1417, 1010, 967, 842, 768; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.45 (1H, s, –NH), 8.02 (4H, m, Ar–H), 7.97 (2H, d, $J = 15.6$ Hz), 7.90 (1H, d, $J = 12.3$ Hz), 7.80 (3H, m, Ar–H), 6.35 (1H, s, Ar–H), 3.97 (6H, s, $-OCH_3 \times 2$), 3.85 (3H, s, $-OCH_3$). MS: m/e 566 (M + 2). *Anal.* Calcd. For $C_{26}H_{21}BrF_3NO_5$: C 55.33, H 3.75, Br 14.16, F 10.10, N 2.48, O 14.18. Found: C 55.36, H 3.77, Br 14.19, F 10.13, N 2.51, O 14.21.

*N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl)acryloyl) phenyl)-3-trifluorobenzamide (**5c**)* (yield 53%); mp 131–133°C; IR υ_{max} cm^{-1} (KBr): 3537, 3103, 3078, 2970, 1659, 1616, 1608, 1583, 1548, 1429, 1398, 1015, 910, 806, 758; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.47 (1H, s, –NH), 8.45 (1H, s, bs), 8.45 (1H, d, $J = 1.4$ Hz), 8.30 (1H, d, $J = 1.6$ Hz), 8.29 (1H, t), 7.84 (1H, d, $J = 3.4$ Hz), 7.97 (2H, d, $J = 15.6$ Hz), 7.80 (3H, m, Ar–H), 6.35 (1H, s, Ar–H), 3.97 (6H, s, $-OCH_3 \times 2$), 3.85 (3H, s, $-OCH_3$). MS: m/e 566 (M + 2). *Anal.* Calcd. For $C_{26}H_{21}BrF_3NO_5$: C 55.33, H 3.75, Br 14.16, F 10.10, N 2.48, O 14.18. Found: C 55.36, H 3.77, Br 14.19, F 10.13, N 2.51, O 14.21.

*N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl)acryloyl) phenyl)-3-chlorobenzamide (**5d**)* (yield 48%); mp 159–161°C; IR υ_{max} cm^{-1} (KBr): 3415, 3180, 2984, 2874, 1622, 1609, 1538, 1507, 1438, 1421, 1009, 982, 867, 741; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.30 (1H, s, –NH), 8.17 (1H, s), 8.10 (1H, d, $J = 3.4$ Hz), 8.02 (2H, d, $J = 8.5$ Hz), 7.97 (1H, d, $J = 15.4$ Hz), 7.82 (2H, d, $J = 4.3$ Hz), 7.67 (1H, d, $J = 7.8$ Hz), 7.51 (1H, t, Ar–H), 7.36 (1H, t, Ar–H), 6.34 (1H, s, Ar–H), 3.97 (6H, s, $-OCH_3 \times 2$), 3.83 (3H, s, $-OCH_3$). MS: m/e 531 (M + 1). *Anal.* Calcd. For $C_{25}H_{21}BrClNO_5$: C 56.57, H 3.99, Br 15.05, Cl 6.68, N 2.64, O 15.07. Found: C 56.61, H 4.01, Br 15.07, Cl 6.71, N 2.68, O 15.10.

*3-bromo-N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl) acryloyl)phenyl)benzamide (**5e**)* (yield 47%); mp 150–152°C; IR υ_{max} cm^{-1} (KBr): 3315, 3145, 2989, 2842, 1621, 1614, 1598, 1547, 1420, 1410, 1007, 972, 816, 768; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.30 (1H, s, –NH), 8.18 (1H, s, Ar–H), 8.10 (1H, d, $J = 3.6$ Hz), 8.05 (2H, d, $J = 8.7$ Hz), 7.97 (1H, d, $J = 15.6$ Hz), 7.82 (2H, d, $J = 4.5$ Hz), 7.67 (1H, d, $J = 7.8$ Hz), 7.51 (1H, t, Ar–H), 7.36 (1H, t, Ar–H), 6.34 (1H, s, Ar–H), 3.97 (6H, s, $-OCH_3 \times 2$), 3.83 (3H, s, $-OCH_3$). MS: m/e 576 (M + 1). *Anal.* Calcd. For $C_{25}H_{21}Br_2NO_5$: C 52.20, H 3.68, Br 27.78, N 2.43, O 13.91. Found: C 52.23, H 3.71, Br 27.82, N 2.46, O 13.94.

*N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl)acryloyl) phenyl)-4-fluorobenzamide (**5f**)* (yield 40%); mp 63–65°C; IR υ_{max} cm^{-1} (KBr): 3320, 3168, 2989, 2856, 1612, 1618, 1602, 1567, 1410, 1010, 945, 822, 768; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.29 (1H, s, –NH), 8.13 (2H, dd, $J = 15.8$ Hz), 7.99 (2H, dd, $J = 5.86$ Hz), 7.77 (2H, d, $J = 7.74$ Hz), 7.49 (1H, t, Ar–H), 7.26 (1H, s, Ar–H), 7.15 (2H, t), 6.37 (1H, s, Ar–H), 3.70 (6H, s, $-OCH_3 \times 2$), 3.49 (3H, s, $-OCH_3$). MS: m/e 515 (M + 1). *Anal.* Calcd. For $C_{25}H_{21}BrFNO_5$: C 58.38, H 4.12, Br 15.54, F 3.69, N 2.72, O 15.55. Found: C 58.42, H 4.14, Br 15.57, F 3.73, N 2.75, O 15.58.

*4-tertbutyl-N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl) acryloyl)phenyl)benzamide (**5g**)* (yield 47%); mp 75–77°C; IR υ_{max} cm^{-1} (KBr): 3553, 3061, 2958, 1649, 1618, 1585, 1543, 1458, 1410, 1016, 918, 850, 761; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.14 (1H, s, –NH), 8.02 (4H, m, Ar–H), 7.34

(2H, d, $J = 7.8$ Hz), 7.29 (4H, m, Ar–H), 6.36 (1H, s, Ar–H), 3.98 (6H, s, $–OCH_3$), 3.85 (3H, s, $–OCH_3$), 1.35 (9H, s, $–CH_3 \times 3$). MS: m/e 554 (M + 1). *Anal.* Calcd. For $C_{29}H_{30}BrNO_5$: C 63.05, H 5.47, Br 14.46, N 2.54, O 14.48. Found: C 63.08, H 5.51, Br 14.49, N 2.58, O 14.52.

N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl)acryloyl) phenyl)-2-fluorobenzamide (***5h***) (yield 54%); mp 131–133°C; IR υ_{max} cm^{-1} (KBr): 3415, 3115, 2984, 1617, 1610, 1590, 1538, 1468, 1420, 1016, 982, 828, 760; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.61 (1H, d, $J = 15.9$ Hz), 8.22 (1H, d, $J = 8.1$ Hz), 8.14 (1H, s, –NH), 8.06 (1H, d, $J = 18.9$ Hz), 8.00 (1H, d, $J = 15.9$ Hz), 7.82 (1H, d, $J = 7.8$ Hz), 7.54 (3H, m, Ar–H), 7.35 (1H, d, $J = 4.6$ Hz), 7.23 (1H, d, $J = 4.5$ Hz), 6.38 (1H, s, Ar–H), 3.99 (6H, s, $–OCH_3 \times 2$), 3.87 (3H, s, $–OCH_3$). MS: m/e 516 (M + 2). *Anal.* Calcd. For $C_{25}H_{21}BrFNO_5$: C 58.38, H 4.12, Br 15.54, F 3.69, N 2.72, O 15.55. Found: C 58.42, H 4.14, Br 15.57, F 3.73, N 2.75, O 15.58.

N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl)acryloyl) phenyl)-3-fluorobenzamide (***5i***) (yield 54%); mp 106–108°C; IR υ_{max} cm^{-1} (KBr): 3450, 3160, 2965, 1625, 1602, 1587, 1507, 1468, 1407, 1009, 925, 816, 720; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.30 (1H, s, –NH), 8.17 (1H, s), 8.10 (1H, d, $J = 3.6$ Hz), 8.05 (2H, d, $J = 8.7$ Hz), 7.97 (1H, d, $J = 15.6$ Hz), 7.82 (2H, d, $J = 4.5$ Hz), 7.67 (1H, d, $J = 7.8$ Hz), 7.51 (1H, t, Ar–H), 7.36 (1H, t, Ar–H), 6.34 (1H, s, Ar–H), 3.97 (6H, s, $–OCH_3 \times 2$), 3.83 (3H, s, $–OCH_3$). MS: m/e 516 (M + 2). *Anal.* Calcd. For $C_{25}H_{21}BrFNO_5$: C 58.38, H 4.12, Br 15.54, F 3.69, N 2.72, O 15.55. Found: C 58.42, H 4.14, Br 15.57, F 3.73, N 2.75, O 15.58.

N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl)acryloyl) phenyl)-3-methylbenzamide (***5j***) (yield 82%); mp 122–124°C; IR υ_{max} cm^{-1} (KBr): 3420, 3130, 2974, 1620, 1609, 1587, 1538, 1478, 1420, 1012, 925, 847, 735; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 9.49 (1H, s, –NH), 8.10 (1H, s, Ar–H), 7.92 (2H, d, $J = 7.8$ Hz), 7.74 (3H, m, Ar–H), 7.32 (4H, m, Ar–H), 6.35 (1H, s, Ar–H), 3.96 (6H, s, $–OCH_3 \times 2$), 3.90 (3H, s, $–OCH_3$), 2.33 (3H, s, $–CH_3$). MS: m/e 514 (M + 4). *Anal.* Calcd. For $C_{26}H_{24}BrNO_5$: C 61.19, H 4.47, Br 15.66, N 2.74, O 15.67. Found: C 61.22, H 4.52, Br 15.69, N 2.77, O 15.70.

N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl)acryloyl) phenyl)-2-methylbenzamide (***5k***) (yield 43%); mp 137–139°C; IR υ_{max} cm^{-1} (KBr): 3417, 3140, 2964, 1638, 1615, 1590, 1510, 1465, 1415, 1012, 976, 802, 709; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.23 (1H, d, $J = 1.9$ Hz), 8.20 (1H, t, Ar–H), 8.10 (1H, t, Ar–H), 8.10 (1H, s, –NH), 8.08 (1H, d, $J = 1.7$ Hz), 7.92 (2H, d, $J = 7.7$ Hz), 7.32 (4H, m, Ar–H), 6.35 (1H, s, Ar–H), 3.97 (6H, s, $–OCH_3 \times 2$), 3.90 (3H, s, $–OCH_3$), 2.34 (3H, s, CH_3). MS: m/e 514 (M + 4). *Anal.* Calcd. For $C_{26}H_{24}BrNO_5$: C 61.19, H 4.47, Br 15.66, N 2.74, O 15.67. Found: C 61.22, H 4.52, Br 15.69, N 2.774, O 15.70.

N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl)acryloyl) phenyl)-4-methoxybenzamide (***5l***) (yield 53%); mp 105–107°C; IR υ_{max} cm^{-1} (KBr): 3415, 3140, 2964, 1620, 1604, 1588, 1465, 1420, 1012, 970, 709; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.13 (2H, d, $J = 7.7$ Hz), 8.02 (2H, d, $J = 16.4$ Hz), 7.88 (2H, d, $J = 5.5$ Hz), 7.76 (2H, d, $J = 7.6$), 7.26 (1H, s, –NH), 6.96 (2H, d, $J = 8.4$ Hz), 6.33 (1H, s, Ar–H), 3.96 (6H, s, $–OCH_3 \times 2$), 3.90 (3H, s, $–OCH_3$), 3.87 (3H, s, $–OCH_3$). MS: m/e 527 (M + 1). *Anal.* Calcd. For $C_{26}H_{24}BrNO_6$: C 59.33, H 4.60, Br 15.18, N 2.66, O 18.24. Found: C 59.36, H 4.63, Br 15.21, N 2.69, O 18.28.

N-(3-((E)-3-(3-bromo-2,4,6-trimethoxyphenyl)acryloyl) phenyl)thiophene-2-carboxamide (***5m***) (yield 50%); mp 79-81°C; IR υ_{max} cm^{-1} (KBr): 3317, 3005, 2937, 1651, 1610, 1581, 1545, 1462, 1429, 1012, 916, 860, 804; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ; 8.05 (4H, m, Ar–H), 7.76 (2H, d, $J = 7.4$ Hz), 7.49 (2H, d, $J = 4.89$ Hz), 7.38 (1H, s, –NH), 7.12 (1H, s, Ar–H), 6.33 (1H, s, Ar–H), 3.95 (6H, s, $–OCH_3 \times 2$), 3.82 (3H, s, $–OCH_3$). MS: m/e 504 (M + 2). *Anal.* Calcd. For $C_{23}H_{20}BrNO_5S$: C 54.99, H 4.01, Br 15.91, N 2.79, O 15.92, S 6.38. Found: C 55.01, H 4.03, Br 15.93, N 2.82, O 15.95, S 6.41.

TNF-α and IL-6 inhibition assay

Pro-inflammatory cytokine production by lipopolysaccharide (LPS) in THP-1 cells was measured according to the method described by Hwang *et al.* (1933). In brief, THP-1 cells were cultured in RPMI 1640 culture medium (Gibco BRL, Pasley, UK) containing 100 U/ml penicillin and 100 mg/ml streptomycin (100× solutions, Sigma Chemical Co. St. Louis, MO) containing 10% foetal bovine serum (FBS, JRH). Cells were differentiated with phorbol myristate acetate (PMA, Sigma). Following cell plating, the test compounds or vehicle (0.5% DMSO) was added to each well and the plate was incubated for 30 min at 37°C. Finally, LPS (*Escherichia coli* 127:B8, Sigma Chemical Co., St. Louis, MO) was added, at a final concentration of 1 μg/ml. Plates were incubated at 37°C for 24 h, 5% CO_2. Supernatants were harvested and assayed for TNF-α and IL-6 by ELISA as described by the manufacturer (BD Biosciences). The cells were simultaneously evaluated for Cytotoxicity using CCK-8 from Dojindo Laboratories. Percent inhibition of cytokine release compared with the control was calculated (Hwang *et al.*, 1993).

COX-1 and 2 inhibition microtitre assay

The COX-1 and -2 inhibition assay was performed as per the assay protocol instructions of 'Colorimetric COX (ovine) inhibitor Screening Assay Kit', Cayman Chemical

Company, MI, USA. The reaction mixture of 100% initial activity wells contained 160 µl of assay buffer, 150 µl of haem and 10 µl of either COX-1 or 2 enzyme solutions. While the reaction mixture of inhibitor wells was comprised of 150 µl of assay buffer, 10 µl of haem and 10 µl of either enzyme COX-1 or -2, 10 µl of the test samples (1 mM). The plates were carefully shaken for 5 s and were incubated for 5 min at 25°C. After 5 min incubation, 20 µl of the colorimetric substrate solution was added to all the wells, followed by the addition of 20 µl of arachidonic acid to all the wells. The plates were shaken gently for few seconds and again incubated for 5 min at 25°C. The absorbance of all the wells was read at 590 nm using Thermo make Automatic Ex-Microplate Reader (M 51118170). Aspirin and SC560 (1 mM) were tested simultaneously as standard COX-1 and -2 inhibitors, respectively. The COX inhibition activity (%) was calculated using following formula:

$$\text{COX inhibition activity}(\%) = 1 - \frac{T}{C} \times 100$$

where T is the absorbance of the inhibitor well at 590 nM, C is the absorbance of the 100% initial activity without inhibitor well at 590 nM (Bandgar *et al.*, 2010).

Trypsin inhibition assay

The method is based on the measurement of inhibition of trypsin induced hydrolysis of bovine serum albumin (BSA). Trypsin (0.075 mg/ml) was initially incubated with 1 mM individual concentrations of test sample of 0.1 ml for 20 min. The substrate BSA (6 g/100 ml, in 0.1 M phosphate buffer, pH 7.6) was added after 20 min. The reaction mixture was incubated for 25 min at 37°C. The reaction was terminated by the addition 3 ml of CCl_3COOH (5%, w/v). The acid soluble fractions were obtained by centrifuging the contents at 5,000 RPM for 15 min. The amount of protein in the acid soluble fractions was estimated by a method of Salicylic acid (10^{-6} M) was used as a reference drug (Tandon *et al.*, 1982; Lowry *et al.*, 1951).

β-glucuronidase inhibition assay

The effect of the selected compounds on activity of β-glucuronidase was studied using a method described by Demetrios (1998). One millimolar concentration of test sample (0.1 ml) in 0.1 M acetate buffer pH 7.4 for 5 min at 37°C were preincubated with 0.8 ml of 2.5 mM *p*-nitrophenyl-β-D-glucopyranosiduronic acid and 0.1 ml of β-glucuronidase was added. The mixture was incubated for 30 min. Reaction was terminated by addition of 2 ml of 0.5 N NaOH. The reaction mixtures were observed spectrophotometrically at 410 nm. Salicylic acid (1 mM) was used as a reference compound (Gacche and Dhole, 2006).

Anti-cancer activity using propidium iodide fluorescence assay

The selected cancer cell lines such as ACHN (human renal cell carcinoma), Panc 1 (human pancreatic carcinoma), Calu 1 (human non small cell lung carcinoma), H460 (human non cell lung carcinoma) and HCT 116 (human colon carcinoma) were used for evaluation of cytotoxic effect of selected curcumin analogues. The fluorescence signal intensity of the propidium iodide (PI) is directly proportional to the amount of DNA in each cell, PI is not able to penetrate an intact membrane, and so cells must first be permeabilized. Seed cells of 3,000–7,500 cells/well were placed in 200 µl of tissue culture grade 96-well plates and allowed them to recover for 24 h in humidified 5% CO_2 incubator at 37°C. After culturing for 24 h, compounds (in 0.1% DMSO) were added into triplicate wells with 10 µM concentrations. 0.1% DMSO alone was used as control. After 48 h in humidified 5% CO_2 incubator at 37°C condition, the medium was removed and treated with 25 µl of PI (50 µg/ml in water/medium) per well. The plates were freeze at -80°C for 24 h then thawed and allowed it to come at room temperature, and the plate absorbance was read on Fluorometer (Polar-Star BMG Tech), using 530 nM excitation and 620 nM emission wavelength. Lastly, percent Cytotoxicity of the compounds was calculated by using following formula (Dengler *et al.*, 1995):

$$\text{Cytotoxicity}(\%) = 1 - \frac{T}{C} \times 100$$

where T is the O. D. of treated cells and C is the O. D. of control.

The results were compared with the standard anti-cancer drug inhibitors flavopiridol (700 nM) and gemcitabine (500 nM).

DPPH radical scavenging assay

The reaction mixture contained 1 mM concentrations of individual test sample (in absolute ethanol) and DPPH radical (10^{-4} M in absolute ethanol) solution. The contents of the reaction mixture were observed spectrophotometrically at 517 nm after 20 min. Gallic acid (1 mM) was used as a reference drug (Bartolome *et al.*, 2004).

OH radical scavenging activity

OH radicals were generated by using the Ferric ion (Fe^{3+})/ascorbic acid reaction system. The detection of OH radicals

was carried out by measuring the amount of formaldehyde generated from the oxidation of dimethyl sulfoxide. The reaction cocktail contained 0.1 mM EDTA, 167 mM Fe^{3+}, 33 mM DMSO in phosphate buffer of 50 mM pH 7.4. 0.1 ml individual compound (1 mM) solution. Ascorbic acid (150 µl, 10 mM in phosphate buffer) was added finally to initiate the reaction. Trichloroacetic acid (17%, w/v) was used to terminate the reaction. The contents were observed spectrophotomertically at 412 nm for the detection of formaldehyde. Ascorbic acid (1 mM) was used as a reference compound (45.85%) for comparative study (Nash, 1953).

Reducing activity assay

Reduction of Fe^{3+} of $K_3Fe(CN)_6$ to Fe^{2+} by anti-oxidants is the underlying mechanism of the reducing activity assay. The reducing potential of selected compounds was calculated by the fall in extinction of $K_3Fe(CN)_6$ at 420 nm against appropriate blank. The reaction mixture contained 500 ml solution of individual sample (1 mM in 0.5% v/v dimethyl sulfoxide) in 3 ml of 1 mM potassium ferricynide solution and the absorbance was recorded at 420 nm after 10 min reaction time. Ascorbic acid (1 mM) was used as a standard reducing agent (Sasaki *et al.*, 1991).

Conclusion

In this study, we have synthesized a series of novel curcumin analogues and evaluated for anti-inflammatory, anticancer and anti-oxidant activity. Compounds **5f**, **5j** and **5m** were excellent inhibitors of TNF-α and IL-6. Compounds **5c**, **5e**, **5b**, and **5d** showed potent COX-2 inhibition, compounds **5d** and **5f** have shown good trypsin inhibition and compounds **5e**, **5g**, and **5c** exhibited excellent β-glucuronidase inhibition. Compounds **5l** and **5m** showed potent anti-cancer activity against selected five human cancer cell lines and compounds **5a** and **5m** were excellent OH radical scavengers. Compounds **5f**, **5j** and **5m** serve as a lead for manoeuvering novel TNF-α and IL-6 inhibitors. Nevertheless, the results of the present investigation also justify the importance **5l** and **5m** as leads for the development of novel and effective anti-cancer agents.

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