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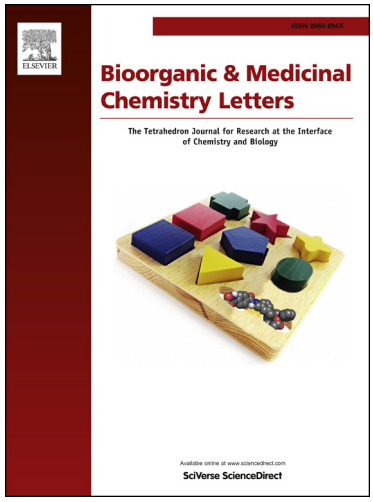

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Synthesis and biological evaluation of new symmetric curcumin derivatives

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Abstract

A series of novel curcumin bisacetamides aiming of enriching their biological activities have been synthesized. The synthesized compounds were screened for their *in vitro* antioxidant, anti-inflammatory and cytotoxic activities. All the compounds exhibited potent to good anti-inflammatory, antioxidant and noteworthy cytotoxic activities.

Keywords: curcumin, acetamides, anti-inflammatory, cytotoxicity, antioxidant.

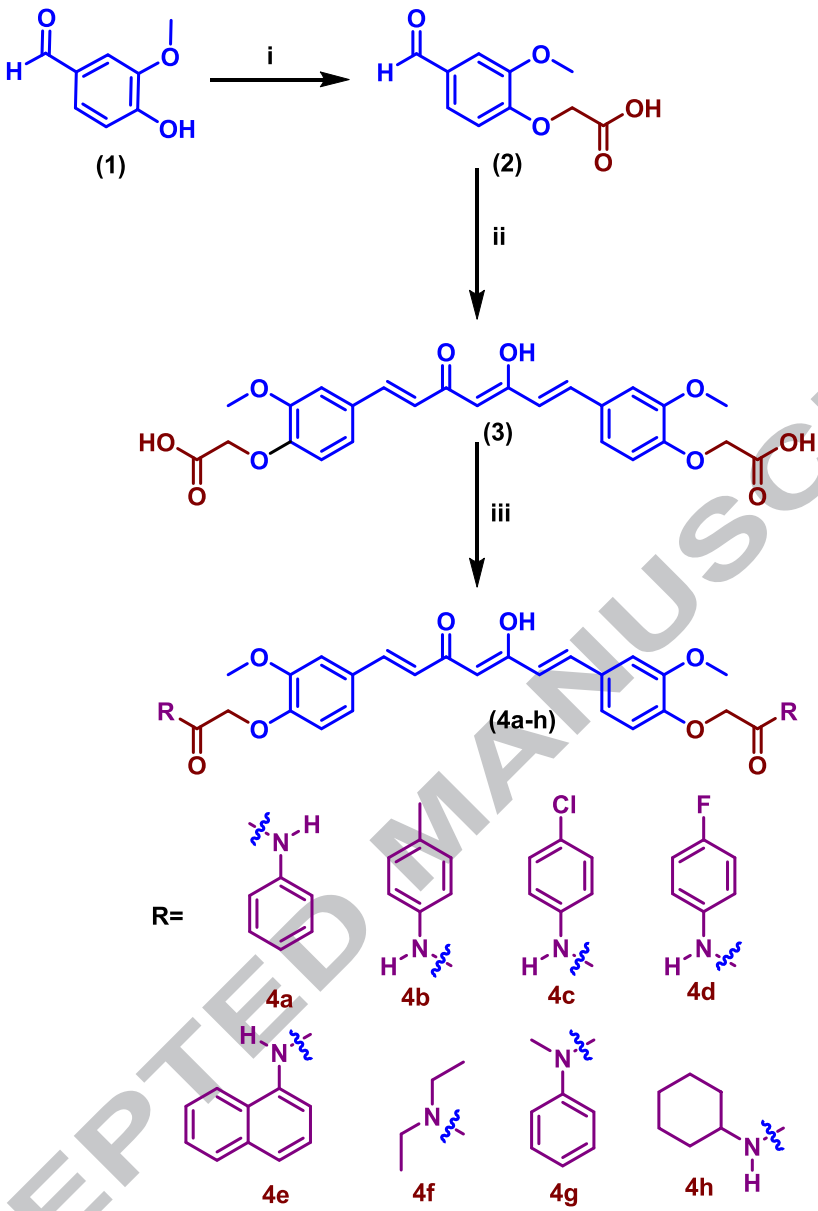
The biological evaluation of natural products and/or their derivatives are fascinating in medicinal research, since most of the natural products are zero toxic. Curcumin is familiar bioactive natural product[1] and food product isolated from curcuma longa Linn[2]. The attention in biomedical research of parent or altered curcumins is due to their extensive spectrum of biological applications[3-10]. The curcumin derivatives have been demonstrated as *in vivo* biologically active with anticancer[11], antidiabetic[12], anti-inflammatory[13] and cytotoxicity[14] characteristics. The significant advantage of the curcumin is that it is nontoxic to human even with high dosage[15]. The clinical trials validate that the daily dose up to 12g for 3 months has not shown any effect on the biological system of healthy people[16].

Even though curcumin is the lead molecule, the researchers are focusing to synthesize covalently modified curcumin. The chemical modification of curcumin can be carried out in several positions (active methylene, aromatic ring and enones) [17-19]. Most of the literature reports has revealed that the phenolic unit of curcumin is responsible for biological activity like free radical scavenging property [20,21]. In addition to that the enolic hydroxyl unit also plays a major role in radical scavenging activity along with phenolic hydroxyl group[22]. Wichitnithad *et al* reported curcuminoids without free phenolic hydroxyl group and have shown enhanced anticancer activity and stability[23]. Ohtsu *et al* reported the cytotoxicity of dimethoxy curcuminoid[24]. Similarly, hindering of hydroxyl group with glucosyl unit in curcumin improved the stability as well as the antioxidant property[25]. Parvathy *et al* reported the curcumin amino acid conjugates on phenolic hydroxyl with enhanced antioxidant activity than the parent drug[26]. Safavy *et al* described

the blocking of hydroxyl with polyethylene glycol which enriched the cytotoxicity[27]. From these intelligences, we have planned to synthesise the curcuminoid blocking the phenolic hydroxyl unit as better bioactive functional group.

One of the well-known simple bioactive functional groups is peptide link which has been demonstrated as a better bioactive molecule in medicinal chemistry. Especially the acetamide or N-substituted acetamides played an essential role in many biological activities[28]. Hence we can anticipate enhanced biological activities by the introduction of acetamido unit to curcumin. Based on this we have introduced the N-substituted acetamido group to the curcumin hydroxyl group and their *in vitro* antioxidant, anti-inflammatory and cytotoxic activities and the details are presented here.

To synthesise the curcumin bisacetamides **(Fig 1)**, the intermediate curcumin bisacetic acid was synthesized as per the reported procedure[29]. Initially the vanillin acetic acid was prepared from vanillin with chloro acetic acid in presence of sodium hydroxide in water[29]. This synthon further used to get boron complex by the conventional method in the presence of acetyl acetone, boron oxide, tri-n-butylborate, n-butyl amine and DMF. The addition of 5% hot acetic acid cleaves the complex and offered the intermediate curcumin bisacetic acid[30]. The coupling of curcumin bisacetic acid with various amines yielded the product[31]. All the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR, mass and IR spectral techniques. The appearance of the amide NH peak in ^{1}H NMR spectrum, the appearance of carbonyl carbon of the amide and the disappearance of acid carbonyl in ^{13}C NMR spectrum indicate the formation of carboxamide. The appearance of a singlet for 6 protons at 4.00 ppm and a singlet for 4 protons at 4.68 ppm indicate the presence of methoxy and methylene (present in between phenoxy and amide) units. The singlet at 5.84 ppm for 1 proton indicates enolic CH. These results revealed that the curcumin is present in the enolic form. The doublet at 6.54 ppm represents the presence of enone CH. Additionally the compound **4a** was characterized by one dimensional DEPT-135 and 2D NMR (^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY and HMBC) experiments. In DEPT-135, 10 positive signals and 1 negative signal were appeared indicating 10 sets of CH and one CH_2 carbons. The correlation of signals at 6.53 & 7.63 in the ^{1}H-^{1}H COSY spectrum clearly indicates the presence of ethylene unit. The signals at 4.00, 4.68 and 5.64 ppm with the signals at 56.2, 70.0 and 101.6ppm respectively in the ^{13}C-^{1}H COSY indicates the presence of O-CH_3, O-CH_2, and enolic CH units. The HMBC correlation of the signals at 4.68ppm with the carbons at 149.00 and 166.4ppm indicates the correlation of acetyl CH with quaternary phenoxy carbon and carbonyl carbon of amide. The contours connecting signals at 6.54 ppm & 130.77 ppm and 6.54 ppm & 183.25 ppm indicate the presence of enone unit. The analytical spectra were shown in the supporting information.



Reagents and condition: (i) $ClCH_2COOH$, NaOH, H_2O,reflux, 12h (ii) 2,4 pentadione, boron oxide, tri n-butyl borate, n-butylamine,70 ^oC, 4h (iii) DCC, HOBT, DMAP, amines, DMF, RT, 24h

Fig. 1. Synthetic route for curcumin bisacetamide derivatives (**4a-h**)

The synthesized curcumin bisacetamides were tested for radical scavenging activity with DPPH free radical[32]. These compounds showed potent radical scavenging ability when compared to the curcumin and vitamin C. Especially the compounds **4c**, **4e** and **4g** having p-chloro acetanilide, napthanilide and N-methylacetanilide moieties have exhibited a better scavenging ability than the curcumin. The compound **4f** and **4h** bearing the aliphatic substituent such as N,N diethyl and cyclohexyl amide disclosed good activity. The compounds **4a**, **4b** and **4d** having acetanilide, p-tolylacetanilide and p-fluoro acetanilide fragments showed

moderate DPPH radical scavenging activity. Overall, curcumin with bulky substituents has showed better activity than the parent and standard. The IC_{50} values of the radical scavenging activity of different compounds are listed in **Table 1**. The scavenging of DPPH free radical by the curcumin bisacetamides could be viewed with naked eye **(Fig 2).** The synthesized curcumin bisacetamides were tested for H_2O_2 scavenging activity as well all compounds showed worthy H_2O_2 scavenging activity than ascorbic acid[33]. The compound **4h** having the cyclohexyl amide unit showed potent H_2O_2 scavenging ability. Similarly compound **4e** bearing naphthalene amide units showed good scavenging activity for which the IC_{50} value is nearer to curcumin. But most of the compounds exhibited less scavenging activity than curcumin but nearer to ascorbic acid. The IC_{50} values of the compounds are listed in **Table 1.**

Table. 1 Antioxidant activity of the compounds **(4a-h)**

S.No	Compound	DPPH(IC_{50}) µg/mL	H_2O_2(IC_{50}) µg/mL
1.	4a	23.77	63.70
2.	4b	22.85	61.98
3.	4c	12.92	65.40
4.	4d	24.26	70.51
5.	4e	12.65	41.40
6.	4f	19.68	68.16
7.	4g	12.05	63.21
8.	4h	16.00	26.14
9.	Curcumin	14.83	34.71
10.	Vitamin C	32.74	67.54

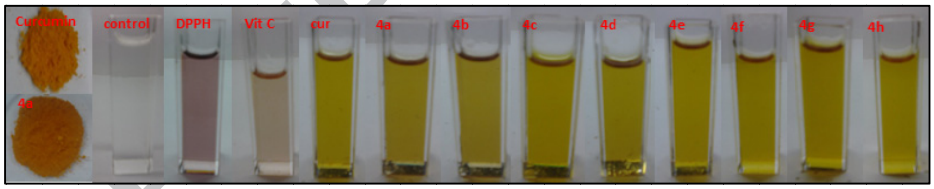
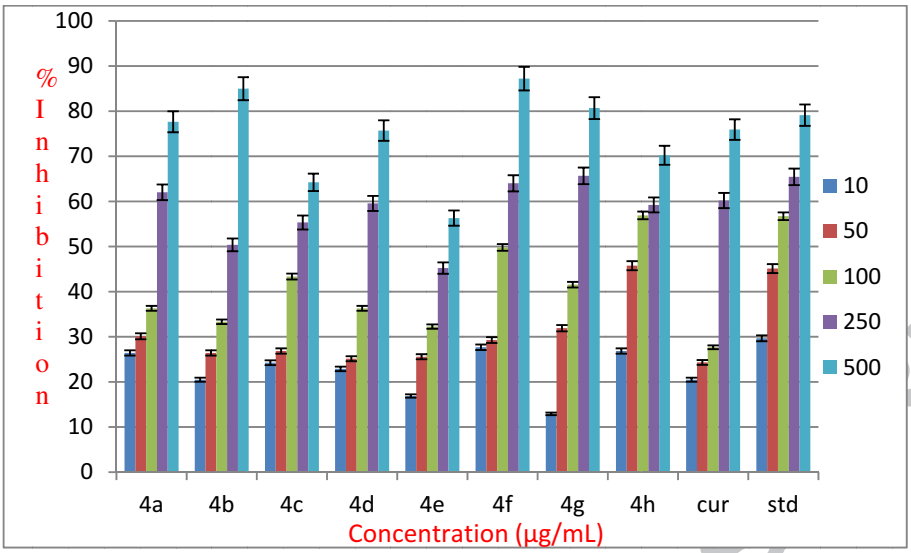


Fig 2. (i) Appearance of curcumin and 4a. (ii) DPPH radical scavenging for the ascorbic acid, curcumin and 4a-h (25µg/mL) with 0.1mM DPPH.

The curcumin bisacetamides were studied for their anti-inflammatory activity (bovine serum albumin assay)[34]. The compounds **4f**, **4g** and **4h** having the N,N-diethyl acetamide, N-methyl acetanilide and cyclohexyl amide showed potent activity than the curcumin, while the compounds **4a**, **4b** and **4d** bearing acetanilide, p-tolylacetmide and p-fluoro acetanilide exhibited good activity. The percentage inhibitions were almost closer to the standards curcumin and diclofenac sodium. The compounds **4c** and **4e** having the p-chloroacetanilide and napthanilide disclosed reasonable activity. Overall, the N-alkyl/cycloalkyl substituted compounds showed enhanced activity with bulky substituents retains the inflammatory activity. The percentage inhibition for the various concentration of synthesized compounds, curcumin and diclofenac sodium are presented in **Fig 3.**



Cur: curcumin, Standard: diclofec sodium

Fig 3. Anti-inflammatory activity of the compounds **4a-h** (Bovine serum albumin assay)

The curcumin bisacetamides were tested for cytotoxic activity by MTT ((3-[4,5-dimethylthiozol-2-yl]-2, 5-diphenyltetrazolium bromide)) assay against A549 cancer cell line[35]. All the synthesized compounds have shown good cytotoxic activity. The compounds **4f** and **4h** having the N,N-diethyl and cyclohexyl acetamide showed very good activity. Other compounds showed only a moderate cytotoxic activity. The experimental results revealed that the compounds having the aliphatic substitution showed good cytotoxic activity than curcumin. The % inhibition for the different concentration of synthesized compounds and curcumins are shown in **Fig 5** and the cell images were represented in **Fig 4.**

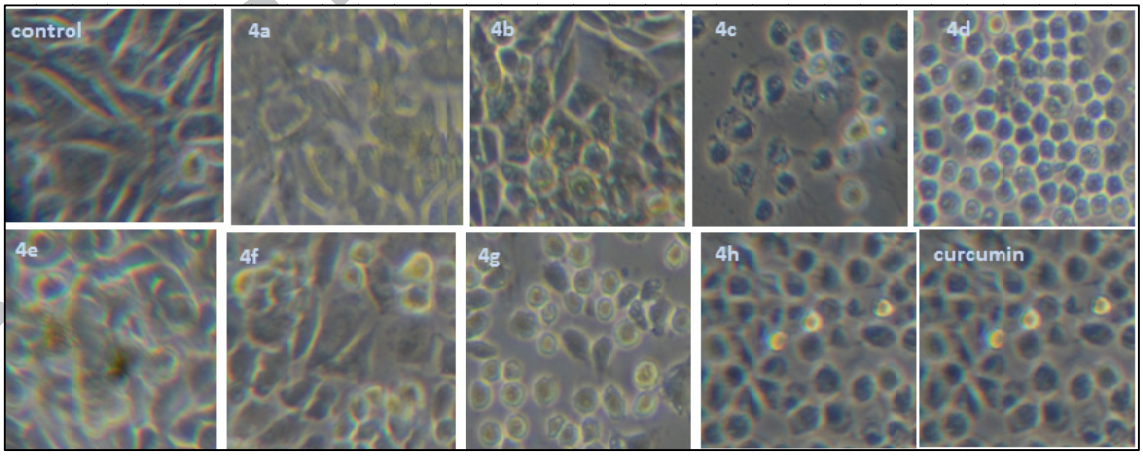
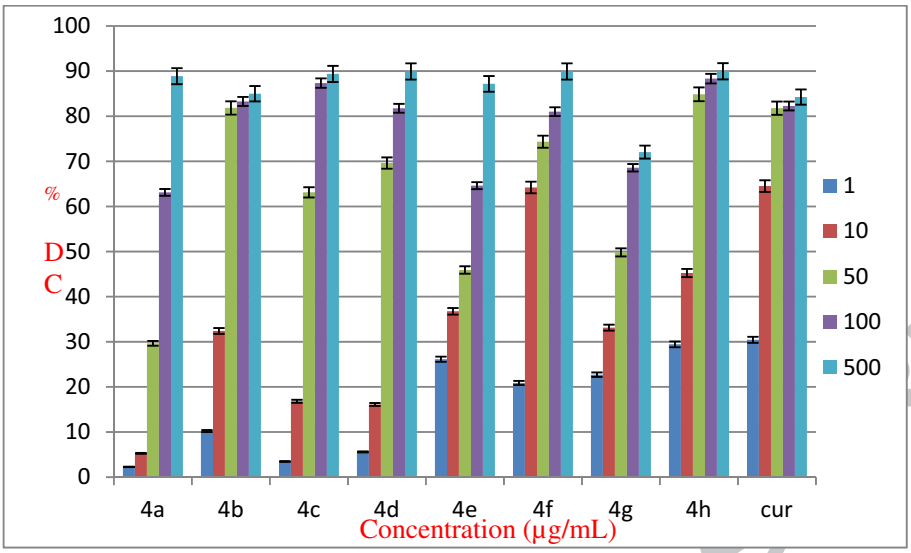


Fig 4. Cell images (A549) of control, **4a-h** (100 µg/mL) **&** Curcumin (100µg/mL)



% DC: % of death cells

Fig 5. Cytotoxic activity of the compounds **4a-h** against A549 cell line

The synthesis of curcumin bisacetamides is reported in this work and it is shown that the introduction of acetamide on the curcumin phenolic hydroxyl enhanced/retained the *in vitro* antioxidant, anti-inflammatory and retained cytotoxic activity. Further work to synthesise water soluble curcumin derivatives with polar units is in progress.

Acknowledgement

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29. Synthetic procedure for 2-(4-formyl-2-methoxyphenoxy)acetic acid **(2):** The vanillin (10g, 0.065 mol) was dissolved in 1N NaOH (160 mL) solution. To that solution chloro aceticacid (7.5g, 0.078 mol) was added and it was refluxed for 12 hrs. Then the reaction mixture was diluted with water (100 mL) washed with ethyl acetate (2 x 75 mL), then the aqueous layer was separated and acidified with Con.HCl. Then the mixture was extracted with ethyl acetate (3 x 100 mL) (in the ethyl acetate layer the product was dissolved along with unreacted vanillin). The combined organic layers were extracted with saturated sodium bicarbonate solution (2 x 75 mL) and separated. The sodium bicarbonate solution was acidified with Con.HCl gave white precipitate of the product. It was filtered and dried in hot air oven at 60 °C for 24hrs. White Solid. Yield: 86.2%. Mp: 176-178 °C IR (KBr Disc) cm-1: 2968, 2924, 1662, 1030, 965. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.86 (s, 1H), 7.49 – 7.39 (m, 2H), 6.92 (d, *J* = 8.6 Hz, 1H), 4.76 (s, 2H), 3.95 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.52, 169.57, 152.52, 130.57, 125.77, 112.14, 109.76, 65.39, 55.74. ESI-MS calculated. m/z 210.04. found 211.05 (M^++1). Anal.Calcd. for: $C_{10}H_{10}O_5$: C, 57.14; H, 4.80% found: C, 57.18; H, 4.78%

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reaction mixture was stirred at 70 °C for 4 hrs. Then the reaction mixture was added to 5% hot acetic acid solution (40 mL) and stirred for 3 hrs. It was diluted with water (100 mL) and extracted with ethyl acetate (2 x 150 mL). The combined organic layers were again washed with water (100 mL) and extracted with 10% sodium bicarbonate solution (2 x 75 mL). The aqueous layer was separated and neutralized with 2N HCl (the PH of the solution should be 6.0-6.5). The yellow precipitate obtained was filtered and dried in vacuum. The filtered solid was washed with ethyl acetate afforded product **3**. Yellow Solid. Yield: 62.5%. Mp: 126-128 °C. IR(KBr Disc) cm-1: 3513, 3068, 2918, 2284, 1710, 1631, 1601, 1030, 966, 808. ^{1}H NMR (300 MHz, DMSO-D_6) δ 7.64 (d, *J* = 15.6 Hz, 2H), 7.43 (s, 2H), 7.29 (d, *J* = 7.8 Hz, 2H), 7.02 – 6.86 (m, 4H), 6.18 (s, 1H), 4.79 (s, 4H), 3.90 (s, 6H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 183.12, 169.83, 149.41, 149.14, 140.15, 128.20, 122.39, 113.09, 111.26, 100.93, 65.17, 55.72. ESI-MS calculated. m/z 484.14. found 485.15 (M^++1). Anal.Calcd. for: $C_{25}H_{24}O_{10}$: C, 61.98; H, 4.99 % found: C, 61.96; H, 4.98;%

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2,2'-((((1E,6E)-3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(N-phenylacetamide)**(4a)**: Yellow solid. Yield: 74.3%. Mp: 160-161 °C. IR (KBr Disc) cm-1: 2960, 2920, 2315, 1697, 1690, 1683, 1030, 976. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.77 (s, 2H), 7.65 – 7.56 (m, 6H), 7.37 (t, J = 7.9 Hz, 4H), 7.20 – 7.12 (m, 6H), 6.98 (d, *J* = 8.3 Hz, 2H), 6.54 (d, *J* = 15.8 Hz, 2H), 5.84 (s, 1H), 4.68 (s, 4H), 4.00 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.26, 166.38, 150.23, 149.00, 140.02, 137.29, 130.78, 129.24, 124.90, 123.55, 122.25, 120.10, 116.12, 111.29, 101.64, 70.05, 56.21. ESI-MS calculated. m/z 634.23. found 635.27 (M^++1). Anal.Calcd. for: $C_{37}H_{34}N_2O_8$: C, 70.02; H, 5.40; N, 4.41% found: C, 70.03; H, 5.39; N, 4.39 %.

2,2'-((((1E,6E)-3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(N-(p-tolyl)acetamide) **(4b)**: Yellow solid. Yield: 74.9%. Mp: 125-126 °C IR (KBr Disc) cm-1: 2961, 2847,

2359, 1683, 1652, 1031, 972. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.72 (s, 2H), 7.59 (d, J = 15.8 Hz, 2H), 7.48 – 7.45 (m, 6H), 7.16-7.10 (m, 4H), 6.95 (d, *J* = 8.3 Hz, 2H), 6.52 (d, *J* = 15.8 Hz, 2H), 5.83 (s, 1H), 4.65 (s, 4H), 3.97 (s, 6H), 2.32 (s, 6H). ^{13}C NMR (75 MHz, CDCl3) δ 183.20, 166.21, 150.01, 148.88, 140.01, 134.54, 130.50, 129.68, 123.32, 122.24, 120.10, 115.75, 111.01, 69.80, 56.09, 20.99. ESI-MS calculated. m/z 662.24. found 663.05 (M^++1). Anal.Calcd. for: $C_{39}H_{38}N_2O_8$: C, 70.68; H, 5.78; N, 4.23 % found: C, 70.66; H, 5.77; N, 4.24 %. 2,2'-((((1E,6E)-3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(N-(4-chlorophenyl)acetamide) (**4c**): Yellow solid. Yield: 73.2%. Mp: 172-174 °C. IR (KBr Disc) cm-1: 2926, 2919, 2849, 1710, 1690, 1652, 1031, 966, 668. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.84 (s, 2H), 7.63 - 7.55 (m, 6H), 7.33 (d, *J* = 8.1 Hz, 4H), 7.18 (d, *J* = 7.7 Hz, 2H), 7.14 (s, 2H), 6.98 (d, *J* = 8.4 Hz, 2H), 6.54 (d, *J* = 15.8 Hz, 2H), 5.85 (s, 1H), 4.68 (s, 4H), 4.00 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.22, 166.47, 150.21, 148.91, 139.96, 135.89, 130.91, 129.93, 129.25, 123.62, 122.26, 121.29, 116.27, 111.38, 70.05, 56.24. ESI-MS calculated. m/z 702.25. found 703.09 (M^++1). Anal.Calcd. for: $C_{37}H_{32}Cl_2N_2O_8$: C, 63.16; H, 4.58; N, 3.98 % found: C, 63.19; H, 4.60; N, 3.95 %.

2,2'-((((1E,6E)-3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(N-(4-fluorophenyl)acetamide) (**4d**): Yellow solid. Yield: 70.2%. Mp: 190-192 °C. IR (KBr Disc) cm-1: 2959, 2920, 2849, 2365, 2340, 1689, 1652, 1028, 968. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.77 (s, 2H), 7.63 – 7.54 (m, 6H), 7.18 (d, *J* = 8.5 Hz, 2H), 7.13 (s, 2H), 7.07 (d, *J* = 8.6 Hz, 4H), 6.98 (d, *J* = 8.3 Hz, 2H), 6.54 (d, *J* = 15.8 Hz, 2H), 5.84 (s, 1H), 4.68 (s, 4H), 3.99 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.23, 166.38, 156.48, 150.07, 148.84, 140.02, 133.25, 130.71, 129.73, 123.47, 122.29, 121.91, 121.81, 117.73, 116.07, 115.77, 111.14, 69.86, 56.17. ESI-MS calculated. m/z 670.21. found 671.10 (M^++1). Anal.Calcd. for: $C_{37}H_{32}F_2N_2O_8$: C, 66.26; H, 4.81; N, 4.18 % found: C, 66.28; H, 4.79; N, 4.19%. 2,2'-((((1E,6E)-3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(N-(naphthalen-1-yl)acetamide) (**4e**): Yellow solid. Yield: 68.7 %. Mp: 195-198°C. IR (KBr Disc) cm-1: 2959, 2922, 2851, 1690, 1631, 1058, 965. ^{1}H NMR (300 MHz, DMSO-D_6) δ 10.11 (s, 2H), 8.09 – 7.94 (m, 4H), 7.86 – 7.75 (m, 6H), 7.66 – 7.50 (m, 8H), 7.33 (d, *J* = 7.6 Hz, 2H), 7.13 (s, 2H), 6.90 (d, *J* = 15.8 Hz, 2H), 6.14 (s, 1H), 4.96 (s, 4H), 3.92 (s, 6H). ^{13}C NMR (75 MHz, DMSO-D_6) δ 183.24, 166.46, 156.47, 149.40, 149.15, 140.15, 134.16, 132.54, 128.68, 128.21, 127.94, 126.30, 126.27, 126.04, 125.68, 122.38, 121.89, 121.23, 113.08, 111.26, 100.94, 70.06, 56.22. ESI-MS calculated. m/z 734.26. found 735.24 (M^++1). Anal.Calcd. for: $C_{45}H_{38}N_2O_8$: C, 73.56; H, 5.21; N, 3.81 % found: C, 73.55; H, 5.20; N, 3.84 %.

2,2'-((((1E,6E)-3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(N,N-diethylacetamide) **(4f)**: Yellow solid. Yield: 65.0%. Mp: 198-200 °C. IR (KBr Disc) cm-1: 2967, 2924, 2851, 1683, 1652, 1029, 966. ^{1}H NMR (300 MHz, CDCl3) δ 7.59 (d, *J* = 15.7 Hz, 2H), 7.12 – 7.08 (m, 4H), 6.93 (d, *J* = 8.1 Hz, 2H), 6.50 (d, *J* = 15.8 Hz, 2H), 5.83 (s, 1H), 4.80 (s, 4H), 3.93 (s, 6H), 3.44 – 3.38 (m, 8H), 1.18 (t, *J* = 6.9 Hz, 6H), 1.15 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.38, 166.83, 149.90, 149.82, 140.38, 129.39, 122.75, 122.36, 114.14, 111.05, 101.34, 68.64, 56.14, 41.71, 40.53, 14.37, 12.91. ESI-MS calculated. m/z 594.29. found 595.46 (M^++1). Anal. Calcd. for: $C_{33}H_{42}N_2O_8$: C, 66.65; H, 7.12; N, 4.71 % found: C, 66.61; H, 7.10; N, 4.74 %.

2,2'-((((1E,6E)-3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(N-methyl-N-phenylacetamide) **(4g)**: Yellow solid. Yield: 69.5%. Mp: 90-92 °C. IR (KBr Disc) cm-1: 2958, 2923, 2854, 1680, 1623, 1045, 965. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 – 7.34 (m, 10H), 7.07 - 7.04 (m, 4H), 7.02 – 6.97 (m, 2H), 6.69 (d, *J* = 8.4 Hz, 2H), 6.47 (d, *J* = 15.8 Hz, 2H), 5.81 (s, 1H), 4.52 (s, 4H), 3.88 (s, 6H), 3.32 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.90, 167.47, 150.02, 149.83, 142.29, 140.64, 130.27, 130.18, 128.56, 127.25, 122.54, 113.81, 110.81, 101.07, 67.22, 56.14, 37.74. ESI-MS calculated. m/z .662.26 found 663.17 (M^++1). Anal.Calcd. for: $C_{39}H_{38}N_2O_8$: C, 70.68; H, 5.78; N, 4.23 % found: C, 70.70; H, 5.75; N, 4.25 %.

2,2'-((((1E,6E)-3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(N-cyclohexylacetamide) **(4h)**: Yellow solid. Yield: 72.3%. Mp: 96-98 °C. IR (KBr Disc) cm-1: 2961, 2917, 2924, 1662, 1645, 1030, 965. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (d, *J* = 15.9 Hz, 2H), 7.16 (d, *J* = 8.2 Hz, 2H), 7.10 (s, 2H), 6.91 (d, *J* = 8.2 Hz, 2H), 6.53 (d, *J* = 15.8 Hz, 2H), 5.84 (s, 1H), 4.55 (s, 4H), 3.95 (s, 6H), 2.36 – 2.28 (m, 2H), 2.08 – 2.06 (m, 4H), 1.83 – 1.79 (m, 4H), 1.56 – 1.41 (m, 8H), 1.38 – 1.19 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 183.26, 167.23, 150.08, 149.19, 140.08, 130.29, 123.30, 122.20, 115.47, 111.17, 101.49, 69.63, 56.05, 47.91, 41.10, 33.02, 24.74. ESI-MS calculated. m/z 646.33. found 645.58 (M^--1). Anal.Calcd. for: $C_{37}H_{46}N_2O_8$: C, 68.71; H, 7.17; N, 4.33 % found: C, 68.70; H, 7.16; N, 4.36 %.

32. DPPH radical scavenging activity: The radical scavenging activity of compound can be determined on the basis of the capacity to scavenge stable 1, 1-diphenyl 2-pieryl hydrazyl (DPPH) radical. The absorbance of DPPH radical is 517 nm in the UV-visible spectrum. The scavenging capacity measured by this method, decrease in the absorbance of DPPH radical, because of the formation of stable DPPH molecule. Curcumin

and ascorbic acid was used as standards. Experiments were repeated as twice. The different concentrations of the compounds are 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL. The stock solution of DPPH (0.1 mmol) in DMSO has been prepared. 2 mL of the stock solution was added to the 2 mL of DMSO and the absorbance has recorded at 517 nm. From the all sample solution, 1 mL was diluted to 2 mL of DMSO and 2 mL of stock solution of DPPH was added and then the absorbance was recorded at 517 nm. The percentage inhibition has been calculated from the blank and tested solution. (**Fig 5**. Evidently displays the scavenging of DPPH radical. The colour of DPPH radical absolutely vanishes with the synthetic compounds). % Inhibition = [(blank-test)/blank] x 100. From the various concentrations of % inhibition, the IC_{50} values are calculated.

33. H_2O_2 scavenging activity: 2.0 mM solution of H_2O_2 was prepared in phosphate buffer (0.2 M, PH = 7.4). The different concentrations of synthesized compounds, curcumin and ascorbic acid were (10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL and 50 µg/mL) added to the hydrogen peroxide solution (0.6 mL) in phosphate buffer. The total solution is made up to 4 mL with phosphate buffer. The same solution without the compound was taken as a negative control. The absorption of hydrogen peroxide recorded at 230 nm and the phosphate buffer was taken as blank. % Inhibition = [(blank-test)/blank] x 100. From the various concentrations of % inhibition, the IC_{50} values are calculated.

34. Anti-inflammatory activity: The synthesized compounds were screened for anti-inflammatory activity by using inhibition of albumin denaturation technique with minor modification. The standard drug and synthesized compounds were dissolved in minimum quantity of Dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, PH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test Solution (4 mL) containing different concentrations of drug was mixed with 1 mL of 1 mM albumin solution in phosphate buffer and incubated at 37 °C in incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in water bath for 15 min. After cooling, the turbidity was measured at 660 nm. Percentage of Inhibition of denaturation was calculated from control where no drug was added. The diclofenac sodium and curcumin were used as standard drug. The percentage inhibition of denaturation was calculated by using following formula. % of Inhibition = 100 x (At - Ac) / At. At = O.D. of test solution. Ac = O.D. of control.

35. Cell culture: Human lung cancer A549 cells were obtained from NCCS Pune, India. Cells were incubated with MEM (Hi Media Laboratories) supplemented with 10% Fetal Bovine Serum, Streptomycin (100 U/ml)

and Penicillin (100U/ml). Cell was maintained in a humidified atmosphere at 37 °C incubator with 5% of CO_2. For treating cells Curcumin bisacetamide were added in DMSO at concentration of 2 mg/ mL and followed by filter sterilization. Then the solution was diluted in growth medium at different concentration 1 µg/mL, 10 µg/mL, 50 µg/mL, 100 µg/mL and 500 µg/mL. Cytotoxicity: The Human lung cells (A549) were seed in 96 well plates in different concentration 1, 10, 50, 100, 500 µg/mL and incubated for 24 hrs and each compound were diluted in 0.1% of DMSO (control). 20 µL of MTT (3-[4,5-dimethylthiozol-2-yl]-2, 5-diphenyltetrazolium bromide) (5mg/mL in phosphate buffer pH = 7.4) was added in each well. After incubation 4 hrs media was carefully removed and purple formazan precipitate was dissolved in 100 µL/well DMSO and kept in incubator for 15 min in dark. Estimation of formazan product was performed at 545 nm in a microplate reader. The assay was performed in triplicate. The data was plotted against the drug concentration (vs) Cytotoxicity.

Synthesis and biological evaluation of new symmetric curcumin derivatives

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Graphical abstract