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RESEARCH PAPER

Design, synthesis, and evaluation of curcumin analogues as potential inhibitors of bacterial sialidase

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ABSTRACT

Sialidases are key virulence factors that remove sialic acid from the host cell surface glycan, unmasking receptors that facilitate bacterial adherence and colonisation. In this study, we developed potential agents for treating bacterial infections caused by *Streptococcus pneumoniae* Nan A that inhibit bacterial sialidase using *Turmeric* and curcumin analogues. Design, synthesis, and structure analysis relationship (SAR) studies have been also described. Evaluation of the synthesised derivatives demonstrated that compound **5e** was the most potent inhibitor of *S. pneumoniae* sialidase ($IC_{50} = 0.2 \pm 0.1\ \mu M$). This compound exhibited a 3.0-fold improvement in inhibitory activity over that of curcumin and displayed competitive inhibition. These results warrant further studies confirming the antipneumococcal activity **5e** and indicated that curcumin derivatives could be potentially used to treat sepsis by bacterial infections.

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Curcumin; sepsis; sialidase; Nan A

1. Introduction

Sepsis, a life-threatening organ dysfunction caused by a dysregulated host response to infection, is caused by an overwhelming immune response to an existing bacterial infection[1]. It commonly occurs in the ageing population and results in ~20–30 million cases annually worldwide. Overall, sepsis remains one of the top five causes of death worldwide[2], also the mortality rate is ~20–50% for severe sepsis and 40–80% for septic shock. Especially, bacterial sepsis is a major cause of mortality of hospitalised patients, thus the development of drugs for bacterial sepsis is urgently needed and many efforts have been undertaken in the medicinal and pharmaceutical industry.

The Gram-positive bacterium, *Streptococcus pneumoniae,* is one of the causes of sepsis. It also major human pathogen and causes a variety of diseases, including bacterial meningitis, otitis media, pneumonia, conjunctivitis[3–6]. Several virulence factors contribute to colonisation and early infection processes, above all sialidases from bacteria are considered key virulence factors[7]. Sialidase removes the terminal sialic acid residues from host cell surface glycans, unmasking receptors that facilitate bacterial adherence and colonisation[8]. This process causes resistance to penicillin and other antibiotics that are used to treat *S. pneumoniae* infection[9]. According to known literature, all clinical isolates of *S. pneumoniae* have sialidases activity known to be involved in sepsis[10]. *S. pneumoniae* sialidase genes in clinical pneumococcal isolates determined that Nan A, Nan B, and Nan C are present in 100%, 96%, and 51% of these strains. Among these sialidases, Nan A has been shown to play an essential role in host–pneumococcal interactions in the respiratory tract and sepsis in mouse models[7,11,12,33]. Therefore, high-affinity inhibitors that can block Nan A are potential agents for prevention and treating sepsis. In the last few years, several studies have reported the discovery of viral or bacterial sialidase inhibitors from an isolated natural product such as flavonoids, coumarins, diplacone, mimulone, pterocarpans, and phlorotannins. However, these compounds are known inhibitors as *Clostridium perfringens* (*Cp*-Nan I) or viral sialidase[9,14–17]. Recently, some studies have reported to inhibition of *S. pneumoniae* sialidase such as diazenylaryl sulphonic acids, malabaricone C, Artocarpin, and anthraquinone glycosides[9,10,18–20]. Therefore, to develop novel bacterial sialidase inhibitors, we focused on the natural product, *Turmeric*, because it had not yet been evaluated.

Turmeric has been used as a traditional medicine for conditions such as liver disease[21], indigestion[22], rheumatoid arthritis[23], and insect bites[24] and is consumed daily by millions of people for the treatment of various diseases. Curcumin is the primary component of *Turmeric* and has a feruloyl methane group containing methoxy, hydroxyl, and heptadienyl with a 1,3-diketone moiety. Curcumin has been extensively studied in the past few decades as an important therapeutic compound. In addition, it still receives a lot of attention for its biological properties, including anti-inflammatory, anti-viral, anti-bacterial, anti-cancer, anti-oxidant, and anti-carcinogenic activities[25], and its use in debilitating diseases such as Crohn's disease, ulcerative colitis[26], and Alzheimer's disease[27,28]. Therefore, many studies evaluating the biological activity of curcumin have been performed and potential curcuminoids have been developed for several diseases.

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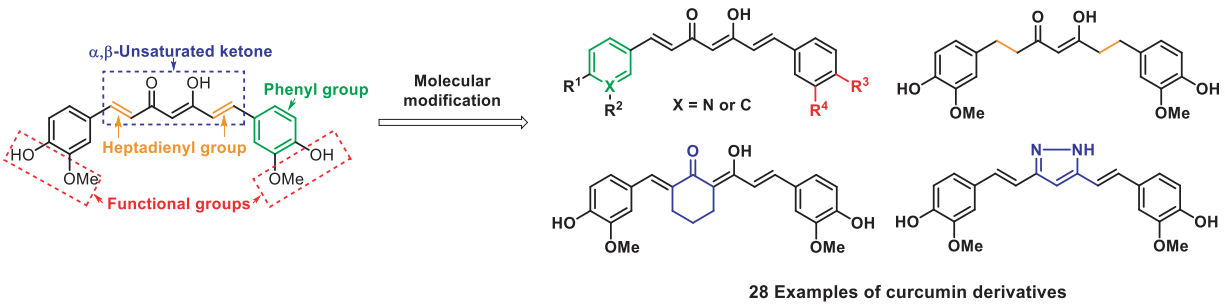


Scheme 1. Designed strategies for the synthesis of curcumin analogues.

In this study, we report that *Turmeric* and curcumin derivatives can targeting the *S. pneumoniae* Nan A. Designed strategies for synthesis of curcumin analogues are shown Scheme 1.

2. Materials and methods

2.1 General

All the chemical reagents used in this work and curcumin (**4a**) were purchased from commercial suppliers (Aldrich, St. Louis, MO; TCI, Japan; Alfa Aesar, Haverhill, MA or Acros Organics, USA companies) and used without further purification. The ^{1}H and ^{13}C NMR spectra were recorded using a JEOL ECA-500 spectrometer, Japan at 500 MHz and 125 MHz, respectively, with chemical shift (δ) values reported in ppm unit. Multiplicities are describes as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), multiplet (m), and broad exchangeable proton (bs). High resolution mass spectra were obtained on a GC Mate 2, JEOL. A CEM Discover system (No. 908005) fitted with a temperature controller was used for microwave reactions. Irradiation was initiated at 300 W to raise the temperature to the set point (150 °C). Reactions were monitored by thin-layer chromatography (TLC) with Merck's DC-Fertigiplatterm Kiegel 60 GE254 plates. Visualisation was accomplished with either UV light or by immersion in a solution of phosphomolybdic acid (PMA) followed by heating on a hot plate for ~10 s. The reaction products were purified by open column chromatography using silica gel produced by Merck (Darmstadt, Germany) (Silica gel 60; 63–200 mesh, ASTM) or Cosmosil 140 C-18 OPN produced by Nacalai Tesque, Inc., USA.

2.2 Expression, purification, and preparation of S. pneumoniae Nan A

We have synthesised and expressed the full-length genes for the *S. pneumoniae* sialidase (Nan A) in *Escherichia coli*. The gene encoding Nan A (Figure S28, GenBank accession no. COT45929.1, PDB: 2VVZ) of *S. pneumoniae* TIGR4 was synthesised (Thermo Fisher Scientific GENEART GmbH, Regensburg, Germany). The synthesised gene was inserted into the cloning sites of a pET151/d-TOPO vector (Invitrogen, Carlsbad, CA) containing a 6x His-tag at the C-terminus. *S. pneumoniae* sialidase was expressed and purified from *E. coli* BL21 (DE3) (HIT; Real biotech Co., Taipei, Taiwan). The purified Nan A was detected at ~56.6 kDa with greater than 90% purity using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Figure S29). The purified sialidase had specific activities (K_m values) of 43.9 μM using 4-methylumbelliferyl-α-*D*-*N*-acetylneuraminic acid sodium salt hydrate (MUNANA; Catalogue No. M8639; Sigma) as substrate (Figure S30).

2.3 Enzyme inhibition activity

As described, the inhibitory effects of compounds on *S. pneumoniae* Nan A were measured using a fluorescence (FL)-based assay. In this assay, the 4-methylumbelliferyl-α-*D*-*N*-acetylneuraminic acid (Sigma Chemical Co., St. Louis, MO) was used as a substrate, and the enzyme activity was determined by measuring the increase in fluorescence by continuously monitoring the reactions at 450/40 nm with excitation at 365 nm using a SpectraMax M2e Multimode Reader (Molecular Devices Co.). The IC_{50} values of the synthesised compounds were measured in a reaction mixture containing enzyme (final concentration of Nan A, 2.2 nM), the test compounds (from 0 to 200 μM), and 50 μM of substrate in 20 mM Sodium phosphate buffer (pH 7.5, containing 300 mM NaCl). To determine the enzyme activity, the experimental data were fit to a logistic curve with Equation (1), a time-drive protocol was used and the initial velocity was recorded over a range of concentrations, and the data were analysed using a nonlinear regression program (Sigma Plot; SPCC Inc., Chicago, IL).

$$\text{Inhibition activity } (\%) = 100 - \left[(S) - (S_0)/(C - C_0)\right] * 100, \quad (1)$$

where C is the fluorescence of the control (enzyme, buffer, and substrate) after 60 min of incubation, C_0 is the fluorescence of the control at 0 min, S is the fluorescence of the tested samples (enzyme, sample solution, and substrate) after incubation, and S_0 is the fluorescence of the tested samples at 0 min.

2.4 Other sialidases assay

Clostridium perfringens sialidase (Catalogue No. 2876; Sigma) and *Vibrio cholerae* sialidase (Catalogue No. 72197; Sigma) activities were evaluated according to a method described by Lee et al.[9] using 4-MUNANA as the substrate, where 10 μL of enzyme solution was mixed with 20 μL of 0.4 mM MUNANA in 20 mM sodium acetate buffer at pH 5.5 with 4 mM $CaCl_2$ 150 mM NaCl. For the inhibition studies, *C. perfringens* sialidase and 0–200 μM of the individual compounds were mixed with MUNANA at 37 °C. The production of 4-methylumbelliferone was measured by monitoring the fluorometric determination at excitation wavelength 365 nm/emission wavelength 450 nm.

2.5. Enzyme kinetic study

The inhibition mechanism was determined, and the apparent inhibition constants (K_i) for the respective sialidase (Nan A) were performed on the test compounds, for which the IC_{50} values were below 25 μM. The test compounds were studied at three different concentrations that were chosen based on the IC_{50} values obtained with each sialidases (~1/2 × IC_{50}, IC_{50}, 2 × IC_{50}).

The concentrations of marker substrates were chosen (~1/4 K_m, 1/2 K_m, K_m) with regard to their Michaelis–Menten kinetics. The K_i values were calculated by nonlinear regression analysis by fitting different models of enzyme inhibition to the kinetic data using SigmaPlot Enzyme Kinetics Module 1.3 (SPSS Inc., Chicago, IL). The inhibition mechanism of the compounds was determined by comparing the statistical results, including the Akaike's information criterion values, of different inhibition models and selecting the one with the best fit.

2.6 General procedures for the synthesis of curcumins and the characterisation of synthesised compounds

2.6.1 Synthesis of curcumin derivatives using Pabon's reaction (compounds 3, 4b, 4e, 5a–5p, 5r, 5s)

Boron trioxide (43.44 mmol) was added to a solution of 2,4-pentadione (65.16 mmol for **3**, **4**) or monophenyl intermediate (**3**, 21.7 mmol) in ethyl acetate (100 ml) at ambient temperature. After stirring for 1 h at 90 °C, the corresponding benzaldehydes (21.7 mmol) and triethyl borate (21.7 mmol) in ethyl acetate were added to the reaction mixture. The mixture was stirred for 2 h at 90 °C, then *n*-$BuNH_2$ (21.7 mmol, 1 equiv., 7% solution in ethyl acetate) was slowly added, and the mixture was stirred at 90 °C until the aldehydes disappeared on TLC monitoring. The reaction mixture was then cooled to 50 °C and 1 M HCl (aq.) was added. After stirring the mixture for an additional 1 h and cooling to room temperature, water (30 ml) and ethyl acetate (20 ml) were added. The reaction mixture was washed with water and brine until it was neutralised. The organic phase was dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was purified using open-bed column chromatography on silica gel or Cosmosil 140 C–18 OPN to yielded curcumin derivatives.

5-Hydroxy-1,7-bis(3-hydroxy-4-methoxyphenyl)hepta-1,4,6-trien-3-one (4b): Yield: 556 mg (23%); orange solid; mp: 182–183 °C (Lit[34]. 181–183 °C); ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.83 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 5.98 (s, 1H), 6.64 (d, 1H, J = 15.5 Hz), 6.73 (d, 1H, J = 15.0 Hz), 6.97 (d, 1H, J = 1.5 Hz), 6.99 (d, 1H, J = 1.5 Hz), 7.11 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 7.17 (d, 1H, J = 1.5 Hz), 7.21 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.31 (d, 1H, J = 2.0 Hz); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.28, 55.46, 101.1, 110.3, 111.5, 111.6, 113.4, 121.7, 122.0, 122.8, 128.1, 128.5, 140.2, 140.3, 146.9, 149.7, 149.8, 151.6, 100.3, 114.1, 115.5, 183.3. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{21}H_{20}O_6$ 368.1260; Found: 368.1261.

5-Hydroxy-1,7-bis(4-hydroxyphenyl)hepta-1,4,6-trien-3-one (4e): Yield: 378 mg (15%); orange solid; mp: 234–235 °C (Lit[35]. 232–233 °C); ^{1}H NMR (500 MHz, Acetone-d_6) δ 5.95 (s, 1H), 6.63 (s, 1H), 6.66 (s, 1H), 6.86–6.89 (m, 4H), 7.53 (s, 2H), 7.55 (s, 2H), 7.56 (s, 1H), 7.59 (s, 1H), 8.99 (bs, 2H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 100.9, 115.9, 121.2, 126.9, 130.1, 140.2, 159.6, 183.7. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{19}H_{16}O_4$ 308.1049; Found: 308.1047.

7-(3,4-Dimethoxyphenyl)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (5a): Yield: 245 mg (50%); dark orange solid; mp: 136–137 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.83 (s, 3H), 3.85 (s, 3H), 3.89 (s, 3H), 5.95 (s, 1H), 6.70 (t, 2H, J = 6.7 Hz), 6.86 (d, 1H, J = 8.0 Hz), 6.97 (d, 1H, J = 8.0 Hz), 7.15 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 7.20 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.30 (dd, 2H, J_1 = 4.7 Hz, J_2 = 1.2 Hz), 7.57 (d, 2H, J = 16.0 Hz), 8.25 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.27, 55.45, 100.9, 110.3, 110.6, 111.6, 122.0, 122.7, 123.0, 128.1, 140.2, 140.7, 147.9, 149.2, 149.7, 151.6, 183.3, 183.9. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{22}H_{22}O_6$ 382.1416; Found: 382.1416.

7-(3,4-Dimethoxyphenyl)-5-hydroxy-1-(3-hydroxy-4-methoxyphenyl)hepta-1,4,6-trien-3-one (5b): Yield: 274 mg (56%); dark orange solid; mp: 155–158 °C (Lit[34]. 157–158 °C); ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.83 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 5.98 (s, 1H), 6.64 (d, 1H, J = 15.5 Hz), 6.73 (d, 1H, J = 15.0 Hz), 6.98 (dd, 2H, J_1 = 8.2 Hz, J_2 = 1.2 Hz), 7.11 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 7.17 (d, 1H, J = 1.5 Hz), 7.21 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.31 (d, 1H, J = 2.0 Hz), 7.54 (d, 1H, J = 16.0 Hz), 7.58 (d, 1H, J = 16.0 Hz), 7.86 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.20, 55.46, 101.1, 110.3, 111.5, 111.6, 113.4, 121.7, 122.0, 122.8, 128.1, 128.5, 140.2, 140.3, 146.9, 149.7, 149.8, 151.6, 183.3. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{22}H_{22}O_6$ 382.1416; Found: 382.1412.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(3-hydroxy-4-methoxyphenyl)hepta-1,4,6-trien-3-one (5c): Yield: 212 mg (45%); red orange solid; mp: 137–140 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.87 (s, 3H), 3.89 (s, 3H), 5.96 (s, 1H), 6.63 (d, 1H, J = 16.0 Hz), 6.69 (d, 1H, J = 15.0 Hz), 6.85 (d, 1H, J = 8.0 Hz), 6.97 (d, 1H, J = 8.5 Hz), 7.10 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.5 Hz), 7.15 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 7.17 (d, 1H, J = 2.5 Hz), 7.32 (d, 1H, J = 1.5 Hz), 7.53 (d, 1H, J = 16.0 Hz), 7.57 (d, 1H, J = 16.0 Hz), 7.83 (bs, 1H), 8.18 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.44, 101.0, 110.9, 111.4, 113.5, 115.3, 121.4, 121.6, 122.0, 123.1, 140.1, 140.7, 146.8, 146.9, 147.9, 149.1, 149.2, 149.7, 183.3, 183.9. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{21}H_{20}O_6$ 368.1260; Found: 368.1262.

1-(3,4-Dihydroxyphenyl)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (5d): Yield: 100 mg (20%); orange solid; mp: 160 °C (decomposed); ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.89 (s, 3H), 5.94 (s, 1H), 6.58 (d, 1H, J = 13.0 Hz), 6.68 (d, 1H, J = 15.5 Hz), 6.86 (s, 2H), 7.03 (s, 1H), 7.15 (d, 1H, J = 4.0 Hz), 7.31 (s, 1H), 7.51 (d, 1H, J = 12.0 Hz), 7.56 (d, 1H, J = 16.0 Hz); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.48, 100.9, 110.6, 114.3, 115.3, 115.6, 121.2, 121.4, 121.8, 123.0, 127.3, 127.5, 140.4, 140.5, 145.5, 147.8, 147.9, 149.1, 183.0, 183.2. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{20}H_{18}O_{16}$ 354.1103; Found: 354.1105.

1-(3,4-Dihydroxyphenyl)-5-hydroxy-7-(3-hydroxy-4-methoxyphenyl)hepta-1,4,6-trien-3-one (5e): Yield: 127 mg (28%); brown solid; mp: 203–204 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.87 (s, 3H), 5.97 (s, 1H), 6.58 (d, 1H, J = 15.5 Hz), 6.64 (d, 1H, J = 16.0 Hz), 6.85 (s, 1H), 6.97 (d, 1H, J = 7.5 Hz), 7.04 (s, 1H), 7.11 (d, 1H, J = 7.0 Hz), 7.18 (s, 2H), 7.51 (d, 1H, J = 15.0 Hz), 7.52 (d, 1H, J = 16.0 Hz); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.46, 101.0, 111.5, 113.4, 115.5, 121.2, 121.6, 121.8, 122.0, 127.4, 128.5, 140.0, 140.6, 145.5, 146.9, 147.8, 149.7, 182.7, 183.6. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{20}H_{18}O_6$ 354.1103; Found: 354.1104.

1-(3-Ethoxy-4-hydroxyphenyl)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (5f): Yield: 284 mg (58%); yellow solid; mp: 182–183 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 1.37 (t, 3H, J = 7.0 Hz) , 3.89 (s, 3H), 4.15 (m, 2H), 5.94 (s, 1H), 6.66 (d, 1H, J = 2.5 Hz), 6.69 (d, 1H, J = 3.0 Hz), 6.85 (d, 2H, J = 8.5 Hz), 7.15 (dd, 2H, J_1 = 10.0 Hz, J_2 = 2.5 Hz), 7.30 (dd, 2H, J_1 = 7.0 Hz, J_2 = 1.5 Hz), 7.54 (d, 1H, J = 3.0 Hz), 7.58 (d, 1H, J = 3.0 Hz), 8.21 (d, 1H, J = 10.5 Hz); ^{13}C NMR (125 MHz, Acetone-d_6) δ 14.64, 55.93, 64.77, 101.3, 111.1, 112.1, 115.8, 121.9, 121.8, 123.3(4), 123.3(6), 123.4(3), 123.4(6), 127.7(4), 127.7(9), 141.0(0), 141.0(8), 147.5, 148.3, 149.5, 149.7, 184.0. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{22}H_{22}O_6$ 382.1416; Found: 382.1417.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(pyridin-3-yl) hepta-1,4,6-trien-3-one (5g): Yield: 33 mg (8%); orange solid; mp: 185 °C (decomposed); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.80 (s, 3H), 6.12 (s, 1H), 6.81–6.84 (m, 2H), 7.15 (d, 1H, J = 8.0 Hz), 7.22 (d, 1H, J = 16.0 Hz), 7.32 (s, 1H), 7.59 (d, 1H, J = 16.0 Hz), 7.66 (d, 1H, J = 16.0 Hz), 7.89 (d, 1H, J = 4.5 Hz), 8.62–8.69 (m, 1H), 8.77 (d, 1H, J = 3.5 Hz), 9.13 (s, 1H), 9.80 (bs, 1H); ^{13}C NMR (125 MHz,

DMSO-d_6) δ 56.23, 102.9, 111.9, 116.2, 121.6, 124.3, 126.5, 126.7, 129.2, 133.8, 141.0, 143.1, 144.4, 144.8, 148.5, 150.4, 179.2, 187.2. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{19}H_{17}NO_4$ 323.1158; Found: 323.1156.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-phenylhepta-1,4,6-trien-3-one (5h): Yield: 45 mg (11%); red brown solid; mp: 135–137 °C (Lit[36]. 137–140); ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.90 (s, 3H), 6.02 (s, 1H), 6.83 (d, 1H, J = 21.5 Hz), 6.86 (d, 1H, J = 13.0 Hz), 7.17 (dd, 1H, J_1 = 8.2 Hz, J_2 = 1.7 Hz), 7.33 (d, 1H, J = 2.5 Hz), 7.38–7.43 (m, 3H), 7.52 (d, 1H, J = 16.0 Hz), 7.63 (d, 1H, J = 8.5 Hz), 7.65 (d, 1H, J = 1.5 Hz), 7.67 (s, 1H), 8.18 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.48, 101.3, 110.7, 115.4, 121.4, 123.2, 124.3, 127.2, 128.1, 129.0, 130.0, 135.3, 139.6, 141.2, 147.9, 149.3, 182.1. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{20}H_{18}O_4$ 322.1205; Found: 322.1205.

Methyl 4-(4-(5-hydroxy-7–(4-hydroxy-3-methoxyphenyl)-3-oxohepta-1,4,6-trien-1-yl)-2-methoxyphenoxy)-butanoate (5i): Yield: 48 mg (8%); yellow solid; mp: 100–101 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 2.04–2.09 (m, 2H), 2.51 (t, 2H, J = 7.2 Hz), 3.61 (s, 3H), 3.85 (s, 3H), 3.88 (s, 3H), 4.07 (t, 2H, J = 6.0 Hz), 5.96 (s, 1H), 6.70 (t, 2H, J = 15.7 Hz), 6.85 (d, 1H, J = 8.0 Hz), 6.97 (d, 1H, J = 7.5 Hz), 7.15 (d, 1H, J = 9.0 Hz), 7.19 (d, 1H, J = 8.0 Hz), 7.30 (s, 2H), 7.57 (d, 1H, J = 16.0 Hz), 8.29 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 24.58, 50.87, 55.37, 55.46, 67.58, 100.9, 110.6(6), 110.6(9), 112.9, 115.4, 121.4, 122.0, 122.7, 123.0, 127.2, 128.3, 140.2, 140.7, 147.9, 149.3, 149.9, 150.7, 172.9, 183.2, 184.0. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{26}H_{28}O_8$ 468.1784; Found: 468.1784.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-nitrophenyl)hepta-1,4,6-trien-3-one (5j): Yield: 249 mg (53%); orange solid; mp: 204–205 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.89 (s, 3H), 6.10 (s, 1H), 6.76 (d, 1H, J = 16.0 Hz), 6.87 (d, 1H, J = 8.5 Hz), 7.05 (d, 1H, J = 16.0 Hz), 7.19 (dd, 2H, J_1 = 8.2 Hz, J_2 = 2.2 Hz), 7.35 (d, 1H, J = 1.5 Hz), 7.65 (d, 1H, J = 16.0 Hz), 7.69 (d, 1H, J = 16.0 Hz), 7.93–7.96 (m, 2H), 8.25–8.28 (m, 2H), 8.31 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.48, 102.2, 110.7, 115.3, 121.6, 123.5, 124.0, 127.0, 128.2, 128.9, 136.3, 141.7, 142.1, 149.9, 148.2, 149.5, 178.8, 186.6. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{20}H_{17}NO_6$ 367.1056; Found: 367.1056.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-(trifluoromethyl)phenyl)hepta-1,4,6-trien-3-one (5k): Yield: 114 mg (23%); yellow solid; mp: 158–160 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.89 (s, 3H), 6.07 (s, 1H), 6.75 (d, 1H, J = 16.5 Hz), 6.86 (d, 1H, J = 7.5 Hz), 6.98 (d, 1H, J = 16.0 Hz), 7.18 (dd, 1H, J_1 = 8.0 Hz, J_2 = 1.5 Hz), 7.34 (d, 1H, J = 1.5 Hz), 7.63 (d, 1H, J = 10.5 Hz), 7.66 (d, 1H, J = 10.5 Hz), 7.75 (d, 1H, J = 7.5 Hz), 7.89 (d, 1H, J = 7.5 Hz), 8.35 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.51, 101.8, 110.8, 115.3, 121.5, 123.4, 125.8, 126.9, 127.1, 128.5, 130.4, 137.3, 139.2, 141.8, 147.9, 149.4, 179.7, 185.9. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{21}H_{17}F_3O_4$ 390.1079; Found: 390.1080.

1-(4-Fluorophenyl)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (5l): Yield: 231 mg (53%); orange solid; mp: 146–147 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.89 (s, 3H), 6.00 (s, 1H), 6.72 (d, 1H, J = 16.0 Hz), 6.79 (d, 1H, J = 16.0 Hz), 6.86 (d, 1H, J = 8.5 Hz), 7.16–7.20 (m, 3H), 7.33 (d, 1H, J = 2.5 Hz), 7.59 (d, 1H, J = 3.0 Hz), 7.62 (d, 1H, J = 3.5 Hz), 7.73 (d, 1H, J = 5.0 Hz), 7.74 (d, 1H, J = 5.0 Hz), 8.27 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.48, 110.7, 115.3, 115.8, 116.0, 121.4, 123.2, 124.2, 127.2, 130.1, 130.2, 130.3, 131.8, 138.2, 141.2, 147.9, 149.2, 162.7, 164.6, 181.4, 184.5. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{20}H_{17}FO_4$ 340.1111; Found: 340.1115.

1-(4-Bromophenyl)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (5m): Yield: 164 mg (32%); yellow solid; mp: 148–149 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.89 (s, 3H), 6.02 (s, 1H), 6.73 (dd, 1H, J_1 = 16.2 Hz, J_2 = 2.2 Hz), 6.85–6.88 (m, 2H), 7.17 (d, 1H, J = 7.5 Hz), 7.33 (s, 1H), 7.56–7.63 (m, 6H), 8.28 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.48, 110.7, 115.3, 110.7, 115.3, 121.5, 123.3, 123.5, 125.1, 127.2, 129.8, 132.1, 134.6, 138.0, 141.4, 147.9, 149.3, 180.7, 185.1. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{20}H_{17}BrO_4$ 400.0310; Found: 430.0309.

***N*-(4–(5-Hydroxy-7–(4-hydroxy-3-methoxyphenyl)-3-oxohepta-1,4,6-trien-1-yl)phenyl)acetamide (5n):** Yield: 281 mg (58%); yellow solid; mp: 183–185 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 2.07 (s, 3H), 3.89 (s, 3H), 5.98 (s, 1H), 6.69 (d, 1H, J = 10.5 Hz), 6.73 (d, 1H, J = 10.5 Hz), 6.86 (d, 1H, J = 8.5 Hz), 7.16 (dd, 1H, J_1 = 7.7 Hz, J_2 = 1.7 Hz), 7.32 (d, 1H, J = 2.5 Hz), 7.57–7.61 (m, 4H), 7.68 (s, 1H), 7.70 (s, 1H), 8.15 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 23.58, 23.62, 55.56, 101.2, 110.7, 115.4, 119.1, 119.2, 121.5, 122.7, 123.2, 127.3, 129.0, 130.1, 139.6, 141.0, 141.4, 141.5, 148.0, 149.3, 168.3, 168.4, 182.8, 184.3. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{22}H_{21}NO_5$ 379.1420; Found: 379.1419.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-methoxyphenyl)hepta-1,4,6-trien-3-one (5o): Yield: 320 mg (66%); yellow solid; mp: 143–145 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.01 (s, 6H), 3.89 (s, 3H), 5.90 (s, 1H), 6.55 (d, 1H, J = 15.0 Hz), 6.67 (d, 1H, J = 16.0 Hz), 6.73 (d, 2H, J = 9.0 Hz), 6.85 (d, 1H, J = 7.5 Hz), 7.14 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.31 (d, 1H, J = 2.0 Hz), 7.50 (d, 1H, J = 9.0 Hz), 7.55 (d, 1H, J = 1.5 Hz), 7.58 (s, 1H), 8.19 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 54.96, 55.46, 101.0, 110.6, 114.4, 115.4, 121.4, 121.9, 123.1, 127.3, 127.8, 129.9, 139.8, 140.7, 147.9, 149.2, 161.5, 183.3, 184.0. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{21}H_{20}O_5$ 352.1311; Found: 352.1314.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)hepta-1,4,6-trien-3-one (5p): Yield: 281 mg (65%); orange solid; mp: 169–170 °C (Lit[37]. 170–172 °C); ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.89 (s, 3H), 5.94 (s, 1H), 6.64 (d, 1H, J = 16.0 Hz), 6.69 (d, 1H, J = 15.5 Hz), 6.84–6.88 (m, 3H), 7.15 (dd, 1H, J_1 = 8.5 Hz, J_2 = 2.5 Hz), 7.31 (d, 1H, J = 1.5 Hz), 7.53–7.56 (m, 3H), 7.59 (d, 1H, J = 3.5 Hz), 8.21 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.45, 101.9, 110.5, 115.4, 115.9, 121.2, 121.4, 123.0, 126.8, 127.3, 130.1, 140.2, 140.5, 147.9, 149.2, 159.7, 183.6. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{20}H_{18}O_5$ 338.1154; Found: 338.1159.

1-(4-(Dimethylamino)phenyl)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (5r): Yield: 220 mg (48%); red solid; mp: 174–175 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.01 (s, 6H), 3.89 (s, 3H), 5.90 (s, 1H), 6.55 (d, 1H, J = 15.0 Hz), 6.67 (d, 1H, J = 16.0 Hz), 6.73 (d, 2H, J = 9.0 Hz), 6.82 (d, 1H, J = 7.5 Hz), 7.14 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.31 (d, 1H, J = 2.0 Hz), 7.50 (d, 2H, J = 9.0 Hz), 7.55 (d, 1H, J = 1.5 Hz), 7.58 (s, 1H), 8.19 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 39.36, 55.52, 100.8, 110.6, 111.9, 115.3, 118.7, 121.5, 122.8, 127.4, 130.0, 139.8, 141.3, 147.8, 148.8, 152.0, 182.0, 184.4. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{22}H_{23}NO_4$ 365.1627; Found: 365.1624.

5-Hydroxy-7-(4-hydroxy-3-methoxyphenyl)-1-(4-(piperidin-1-yl)phenyl)hepta-1,4,6-trien-3-one (5s): Yield: 56 mg (12%); red solid; mp: 204–205 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 1.61–1.63 (m, 6H), 3.22–3.31 (m, 4H), 3.89 (s, 3H), 5.92 (s, 1H), 6.58 (d, 1H, J = 15.5 Hz), 6.67 (d, 1H, J = 15.5 Hz), 6.85 (d, 1H, J = 8.0 Hz), 6.93 (d, 2H, J = 9.5 Hz), 7.14 (dd, 1H, J_1 = 8.2 Hz, J_2 = 1.7 Hz), 7.31 (d, 1H, J = 2.0 Hz), 7.50 (d, 2H, J = 9.5 Hz), 7.54 (d, 1H, J = 5.0 Hz), 7.57 (d, 1H, J = 4.5 Hz), 8.19 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 24.22, 25.36, 48.70, 55.46, 100.81, 110.58, 114.7, 115.3, 119.7, 121.5, 122.9, 124.3, 127.4, 129.8, 140.1, 140.7, 147.9, 149.1, 153.0, 182.9, 184.3. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{25}H_{27}NO_4$ 405.1940; Found: 405.1943.

2.6.2 Synthesis of 1,7-bis(3,4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione (4c)

Curcumin (1.0 g, 2.71 mmol) was dissolved in dry acetone (30 ml) then anhydrous K_2CO_3 (1.12 g, 8.14 mmol) and CH_3I (1.69 ml, 27.14 mmol) were added. The reaction mixture was refluxed for 24 h and monitored by TLC. The reaction mixture was then cooled to ambient temperature and filtrated. The resulting filtrate was evaporated under reduced pressure and ethyl acetate (30 ml) and water (20 ml) were added. The organic layer was washed with water and brine, and then dried over anhydrous $MgSO_4$. After evaporating under reduced pressure, the residue was purified using a Cosmosil 140 C-18 OPN column (CH_3CN:H_2O = 3:2 (v/v)) to yield 1,7-bis(3, 4-dimethoxyphenyl)hepta-1,6-diene-3,5-dione (**4c**, 150 mg, 14%) and 1-(3,4-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (**5a**, 210 mg, 20%) as orange solids.

1,7-Bis(3,4-dimethoxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (4c): Yield: 150 mg (14%); orange solid; mp: 134–136 °C (Lit[38]. 132–133 °C); ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.84 (s, 6H), 3.85 (s, 6H), 5.97 (s, 1H), 6.70 (s, 1H), 6.73 (s, 1H), 6.97 (s, 1H), 6.98 (s, 1H), 7.11 (s, 1H), 7.21 (s, 1H), 7.29 (s, 2H), 7.57 (s, 1H), 7.60 (s, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.30, 101.2, 110.4, 111.6, 122.0, 122.8, 123.0, 123.7, 128.1, 140.4, 142.8, 149.8, 151.6, 183.6. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{23}H_{24}O_6$ 396.1573; Found: 396.1572.

2.6.3 Synthesis of 1,7-bis(3,4-dihydroxyphenyl)hepta-1,6-diene-3,5-dione (4d)[34]

Curcumin (0.3 g, 0.81 mmol) was suspended in dry CH_2Cl_2 (30 ml), then stirred for 10 min at −20 °C under N_2 (g) atmosphere. Tribromoborane (0.25 ml ×5 times) was slowly added. The reaction mixture was allowed to warm up to ambient temperature and stirred overnight. Therefore, the reaction mixture was slowly poured into the saturated $NaHCO_3$ solution (30 ml) with ice powder, and then stirred for 2 h. The water layer was separated and acidified with 1 M HCl (aqueous), then extracted with ethyl acetate (30 ml ×3 times). The organic layer was washed with water and brine, and then dried over anhydrous $MgSO_4$. After evaporating the solvent under reduced pressure, the residue was applied to the top of an open-bed silica gel column (*n*-hexane: ethyl acetate: MeOH =60:38:2 (v/v)) to yield 1,7-bis(3,4-dihydroxyphenyl)hepta-1,6-diene-3,5-dione (**4d**, 27 mg, 10%) as an orange amorphous solid.

1,7-bis(3,4-dihydroxyphenyl)-5-hydroxyhepta-1,4,6-trien-3-one (4d): Yield: 27 mg (10%); orange solid; mp: 300 °C (decomposed, Lit[39]. 304–305); ^{1}H NMR (500 MHz, Acetone-d_6) δ 5.95 (d, 1H, J = 6.0 Hz), 6.58 (dd, 2H, J_1 = 15.5 Hz, J_2 = 6.0 Hz), 6.85 (s, 2H), 7.04 (s, 2H), 7.16 (d, 2H, J = 3.0 Hz), 7.51 (dd, 2H, J_1 = 15.2 Hz, J_2 = 6.2 Hz), 8.39 (bs, 4H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 100.9, 114.4, 115.6, 121.2, 121.8, 127.5, 140.5, 145.5, 147.9, 183.5. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{19}H_{16}O_6$ 340.0947; Found: 340.0949.

2.6.4 Synthesis of 2–(3-hydroxy-4-methoxybenzylidene)-6–(3-(3-hydroxy-4-methoxyphenyl)acryloyl)cyclohexa-n-1-one (4f)

2-Acetyl-cyclohexan-1-one (0.3 g, 2.14 mmol), boron trioxide (0.14 g, 2.14 mmol), morpholine (0.037 ml, 0.42 mmol), acetic acid (0.024 ml, 0.42 mmol), and vanillin (0.651 g, 4.28 mmol) were placed in a capped vial without solvent at ambient temperature. The resulting mixture was irradiated in a microwave oven (300 W output, 1378 KPa) at 150 °C for 40 min until the 2-acetyl-cyclohexan-1-one or vanillin was consumed. The reaction was monitored by TLC. After the reaction vial cooled, the product was dissolved in MeOH. The reaction mixture was evaporated under reduced pressure, then purified further by silica gel column chromatography (*n*-hexane: ethyl acetate: MeOH =60:38:2 (v/v)) to give 2-(3-hydroxy-4-methoxybenzylidene)-6-(3-(3-hydroxy-4-methoxyphenyl)acryloyl)cyclohexan-1-one (**4f**, 390 mg, 45%) as an orange red solid.

2–(1-Hydroxy-3-(4-hydroxy-3-methoxyphenyl)allylidene)-6-(4-hydroxy-3-methoxybenzylidene)cyclohexan-1-one (4f): Yield: 390 mg (45%); orange red solid; mp: 182–183 °C (Lit[40]. 175–176 °C); ^{1}H NMR (500 MHz, MeOH-d_4) δ 1.75–1.80 (m, 2H), 2.67 (t, 2H, J = 6.2 Hz), 2.77 (t, 2H, J = 5.5 Hz), 3.86 (s, 3H), 3.90 (s, 3H), 6.80 (d, 1H, J = 3.5 Hz), 6.82 (d, 1H, J = 4.0 Hz), 6.95 (dd, 1H, J_1 = 8.5 Hz, J_2 = 1.5 Hz), 7.03 (d, 1H, J = 1.5 Hz), 7.07 (d, 1H, J = 15 Hz), 7.13 (dd, 1H, J_1 = 8.2 Hz, J_2 = 2.2 Hz), 7.22 (d, 1H, J = 2.5 Hz), 7.58 (s, 1H), 7.63 (d, 1H, J = 15.5 Hz); ^{13}C NMR (125 MHz, MeOH-d_4) δ 22.90, 24.03, 27.08, 55.06, 108.3, 110.6, 113.5, 114.8, 115.2, 117.3, 122.9, 123.8, 127.4, 128.2, 130.6, 133.2, 142.5, 147.1, 147.4, 148.0, 149.2, 177.6, 185.7. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{24}H_{24}O_6$ 408.1573; Found: 408.1572.

2.6.5 Synthesis of 1-(4-aminophenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1, 6-diene-3,5-dione (5q)

Compound **5n** (100 mg, 0.26 mmol) was dissolved in tetrahydrofuran (5 ml), then ethanol (20 ml) and 1 M HCl (aq., 20 ml) were added. The reaction mixture was stirred at reflux for 22 h and monitored by TLC, then cooled to ambient temperature and evaporated under reduced pressure. Ethyl acetate (20 ml) was added to the residue and the mixture was neutralised using $NaHCO_3$ (aq, sat.). The organic phase was washed with water (20 ml ×3 times) and brine, dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was applied to the top of an open-bed silica gel column (*n*-hexane: ethyl acetate: methanol =60:35:5 (v/v)) to yield 1-(4-aminophenyl)-7-(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione (**5q**, 75 mg, 85%) as a red solid.

1-(4-Aminophenyl)-5-hydroxy-7-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (5q): Yield: 75 mg (85%); red solid; mp: 99–100 °C; ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.89 (s, 3H), 5.29 (bs, 2H), 5.89 (s, 1H), 6.51 (d, 1H, J = 15.5 Hz), 6.65–6.68 (m, 3H), 6.85 (d, 1H, J = 8.5 Hz), 7.13 (dd, 1H, J_1 = 8.0 Hz, J_2 = 2.0 Hz), 7.30 (d, 1H, J = 2.0 Hz), 7.39 (d, 2H, J = 9.5 Hz), 7.52 (d, 1H, J = 2.5 Hz), 7.55 (d, 1H, J = 2.5 Hz), 8.22 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.46, 100.6, 110.5, 114.2, 115.3, 118.6, 120.0, 121.4, 122.9, 123.4, 127.4, 129.2, 130.2, 139.9, 141.4, 147.9, 149.0, 151.3, 182.4, 184.8. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{20}H_{19}NO_4$ 337.1314; Found: 337.1316.

2.6.6 Synthesis of 1,7-bis(3-hydroxy-4-methoxyphenyl)heptane-3,5-dione (6)

Pd-C (10%, 50 mg) was added to a solution of curcumin (0.5 g, 0.27 mmol) in methanol (10 ml). After degassing, the mixture was stirred at 0 °C for 10 min, and then hydrogenated in a hydrogen atmosphere (balloon) for 12 h. The mixture was filtered through a Celite 545 and the solvent was evaporated under reduced pressure. The residue was applied to the top of an open-bed silica gel column (CH_2Cl_2:MeOH gradient elution) to yield 1,7-bis(3-hydroxy-4-methoxyphenyl)heptane-3,5-dione (**6**, 140 mg, 28%) as a white solid.

1,7-Bis(4-hydroxy-3-methoxyphenyl)heptane-3,5-dione (6): Yield: 140 mg (28%); white solid; mp: 94–96 °C (Lit[41]. 95–97 °C); ^{1}H NMR (500 MHz, Acetone-d_6) δ 2.55 (t, 2H, J_1 = 7.5 Hz, J_2 = 8.5 Hz),

2.76–2.80 (m, 6H), 3.78 (s, 6H), 5.62 (s, 1H), 6.59–6.64 (m, 2H), 6.68–6.70 (m, 2H), 6.78 (d, 1H, $J = 1.5$ Hz), 6.81 (d, 1H, $J = 1.5$ Hz); ^{13}C NMR (125 MHz, Acetone-d_6) δ 30.99, 40.03, 45.07, 55.34, 99.49, 111.8, 114.8, 120.6, 132.2, 144.9, 147.5, 197.6. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{21}H_{24}O_6$ 372.1573; Found: 372.1574.

2.6.7 Synthesis of (3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-1,4-phenylene) diacetate (7)

N,*N*-dimethylaminopyridine (0.13 g, 1.08 mmol) was added to a solution of curcumin (2 g, 5.42 mmol) in anhydrous pyridine (10 ml) at ambient temperature. Acetic anhydride (1.02 ml, 10.8 mmol) was dropped into the reaction mixture in a 0 °C ice bath, and then stirred overnight at ambient temperature. The reaction mixture was then poured into crashed ice and extracted with EtOAc (3 × 30 ml). The organic layer was washed with water, dried over anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography (CH_2Cl_2) to yield di-(3,5-dioxohepta-1,6-diene-1,7-diyl)bis(2-methoxy-1,4-phenylene) diacetate[7] (1.9 g, 77%) as a yellow solid.

3-Hydroxy-5-oxohepta-1,3,6-triene-1,7-diyl)bis(2-methoxy-4,1-phenylene) diacetate (7): Yield: 1.9 g (77%); yellow solid; mp: 158–159 °C (Lit[38]. 156–158); ^{1}H NMR (500 MHz, Acetone-d_6) δ 2.23 (s, 6H), 3.86 (s, 6H), 6.04 (s, 1H), 6.82 (s, 1H), 6.86 (s, 1H), 7.08 (s, 1H), 7.10 (s, 1H), 7.24 (d, 1H, $J = 2.5$ Hz), 7.26 (d, 1H, $J = 1.5$ Hz), 7.41 (d, 2H, $J = 2.0$ Hz); ^{13}C NMR (125 MHz, Acetone-d_6) δ 19.65, 55.54, 101.6, 111.7, 121.1, 123.3, 124.4, 134.0, 139.8, 141.7, 151.8, 168.0, 183.6. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{25}H_{24}O_8$ 352.1472; Found: 352.1472.

2.6.8 Synthesis of 4,4'-((1H-pyrazole-3,5-diyl)bis(ethene-1,2-diyl))-bis(2-methoxyphenol) (8)

Hydrazine hydrate (0.07 ml, 0.81 mmol) was added to a solution of curcumin (0.2 g, 0.54 mmol) in glacial acetic acid at room temperature, then the mixture was stirred for 2 h under reflux and the solvent was evaporated under reduced pressure. EtOAc (30 ml) was added to the residue and the organic layer was washed with water and brine, and then dried over anhydrous $MgSO_4$. After the solvent was evaporated under reduced pressure, the residue was applied to the top of an open-bed silica gel column (CH_2Cl_2: MeOH =95:5 (v/v)) to yield 4,4'-((1*H*-pyrazole-3, 5-diyl)bis(ethene-1,2-diyl))bis(2-methoxyphenol) (**8,** 130 mg, 68%) as an ivory solid.

4,4'-(1*H*-Pyrazole-3,5-diyl)bis(ethene-1,2-diyl))bis(2-methoxyphenol) (8): Yield: 130 mg (68%); ivory solid; mp: 220–222 °C (Lit. 218 °C); ^{1}H NMR (500 MHz, Acetone-d_6) δ 3.86 (s, 6H), 6.65 (s, 1H), 6.79 (s, 1H), 6.81 (s, 1H), 6.96 (d, 1H, $J = 3.0$ Hz), 6.98 (d, 1H, $J = 1.5$ Hz), 7.00 (s, 1H), 7.09 (s, 1H), 7.12 (s, 1H), 7.17 (d, 2H, $J = 2.5$ Hz), 8.21 (bs, 1H); ^{13}C NMR (125 MHz, Acetone-d_6) δ 55.38, 99.21, 109.0, 115.2, 115.8, 120.3, 129.2, 129.9, 146.9, 147.1, 147.8. HRMS (EI) *m/z*: $[M]^+$ calcd. for $C_{21}H_{20}N_2O_4$ 364.1423; Found: 364.1423.

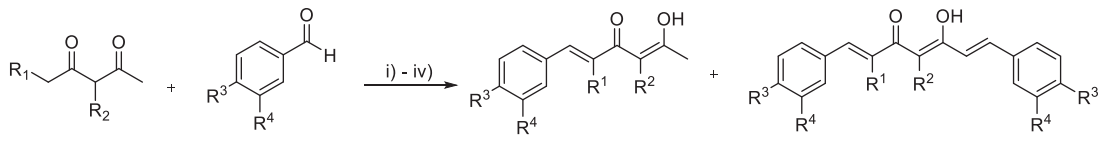
3. Results and discussion

3.1 Chemistry

The synthetic route of the curcumin derivatives is shown in Schemes 2–4. The 6-(3, 4-substituted-phenyl)-hex-5-ene-2,4-diones (**3**) were prepared using acetylacetone with 1 equivalent of corresponding aldehydes by Pabon's method. From this reaction, the symmetrical curcumins, **4a–4e**, were obtained as by-products (Scheme 2)[39]. The symmetrical curcumin derivatives, **4**, were synthesised by combining **1** with 2 equivalents of corresponding aldehydes **2**, except of compounds **4c** and **4d** owing to trace yields. The synthesis of **4c** and **4d** is shown in Scheme 4. Compound **4f**, which has an inserted cyclohexyl group in 1,3-diketone, was prepared by combining 2-acetylcyclohexanone with vanillin under microwave irradiation[40].

Twenty asymmetrical curcumin derivatives, **5**, were prepared using Pabon's reaction with **3** and the corresponding aldehydes (Scheme 3)[39]. Compound **5q** was synthesised by deacetylation of **5n** using 1 M HCl aqueous solution.

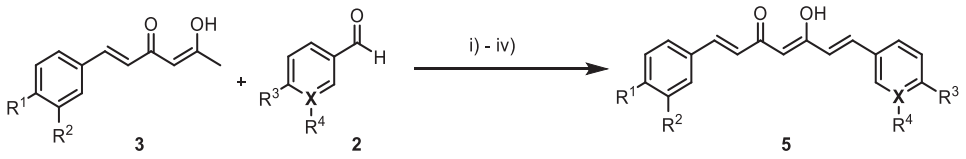
Symmetrical curcumin derivatives **4c**, **4d**, and **6**–**8** were prepared by treating the corresponding reagent with curcumin (Scheme 4). Compound **4c** was prepared with MeI/K_2CO_3 in acetone under reflux conditions and compound **4d** was produced by a demethylation reaction after treating tribromoborane with dry dichloromethane[41]. Tetrahydrocurcumin (**6**) was prepared by hydrogenation using 10% Pd-C as a catalyst[42] and acetylated curcumin **7** was prepared using acetic anhydride with DMAP as a catalyst with anhydrous pyridine[34]. Compound **8** was prepared by condensation the 1, 3-diketone in curcumin with hydrazine hydrate under reflux condition[28]. The structures of all compounds were characterised using ^{1}H NMR, ^{13}C NMR, and EI-HRMS.



3	R^1	R^2	R^3	R^4
3a	H	H	OH	OMe
3b	H	H	OMe	OH
3c	H	H	OMe	OMe
3d	H	H	OH	OH
3e	H	H	OH	H

4	R^1	R^2	R^3	R^4
4a	H	H	OH	OMe
4b	H	H	OMe	OH
4c	H	H	OMe	OMe
4d	H	H	OH	OH
4e	H	H	OH	H
4f	cyclohexyl		OH	OMe

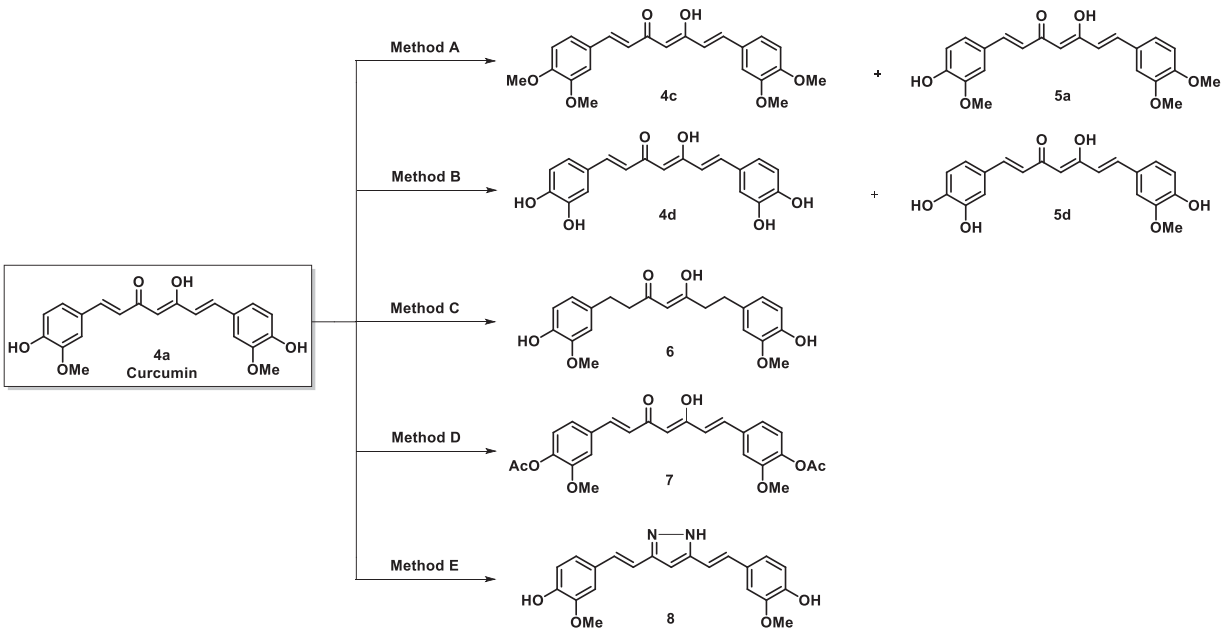
Scheme 2. Reagent and conditions for synthesis of **3:** (i) **1**, B_2O_3, ethyl acetate, 90 °C; (ii) **2**, *n*-$(BuO)_3B$, ethyl acetate, 90 °C; (iii) *n*-$BuNH_2$, ethyl acetate, 90 °C; (iv) hydrochloric acid (1M, aq.), 50 °C; synthesis of **4**: (i) **3**, B_2O_3, ethyl acetate, 90 °C; (ii) **2**, *n*-$(BuO)_3B$, ethyl acetate, 90 °C; (iii) *n*-$BuNH_2$, ethyl acetate, 90 °C; (iv) hydrochloric acid (1M, aq.), 50 °C; synthesis of **4f**: **1**, **2**, B_2O_3, morpholine, AcOH, 40 min, microwave irradiation (300 W).



5	X	R^1	R^2	R^3	R^4	5	X	R^1	R^2	R^3	R^4
5a	C	OMe	OMe	OH	OMe	**5k**	C	OH	OMe	CF_3	H
5b	C	OMe	OMe	OMe	OH	**5l**	C	OH	OMe	F	H
5c	C	OH	OMe	OMe	OH	**5m**	C	OH	OMe	Br	H
5d	C	OH	OMe	OH	OH	**5n**	C	OH	OMe	NHAc	H
5e	C	OMe	OH	OH	OH	**5o**	C	OH	OMe	OMe	H
5f	C	OH	OMe	OH	OEt	**5p**	C	OH	OMe	OH	H
5g	N	OH	OMe	H	H	**5q**	C	OH	OMe	NH_2	H
5h	C	OH	OMe	H	H	**5r**	C	OH	OMe	$N(CH_3)_2$	H
5i	C	OH	OMe	$O(CH_2)_2COOMe$	OMe	**5s**	C	OH	OMe	$c\text{-}C_5H_{10}N$	H
5j	C	OH	OMe	NO_2	H						

1M HCl

Scheme 3. Reagent and conditions for synthesis of asymmetrical curcumin derivatives **5**: (i) **3**, B_2O_3, ethyl acetate, 90 °C; (ii) **2**, *n*-$(BuO)_3B$, ethyl acetate, 90 °C; (iii) *n*-$BuNH_2$, ethyl acetate, 90 °C; (iv) hydrochloric acid (1M, aq.), 50 °C.



Scheme 4. Reagent and conditions for synthesis of curcumin derivatives **4c**, **4d**, and **6**–**8**: (A) **4a**, CH_3I, K_2CO_3, acetone, reflux; (B): **4a**, BBr_3, –78 °C to ambient temperature, N_2 (g), H_2O; (C) **4a**, H_2/Pd-C (10 wt. % of palladium), MeOH, 0 °C to ambient temperature; (D) **4a**, Ac_2O, DMAP, pyridine, ambient temperature; (E) **4a**, $NH_2NH_2{\cdot}H_2O$, AcOH, reflux.

3.2 Biological evaluation

Hydrolytic activity of *S. pneumoniae* Nan A was confirmed using DANA (Neu5Ac2en). The IC_{50} value of DANA with respect to *S. pneumoniae* sialidase inhibition was $4.8 \pm 1.1\ \mu M$[12]. To identify a sialidase inhibitor of *S. pneumoniae*, the inhibitory activity of *Turmeric* ethanol extract and its three major components was compared. For the methanol extract, the Nan A activity was 88% at 30 μg/mL. The sialidase inhibitory activity of the major components of *Turmeric* was as follows; curcumin (**4a**, $IC_{50} = 0.6 \pm 0.1\ \mu M$), demethoxycurcumin (**5p**, $IC_{50} = 0.6 \pm 0.2\ \mu M$), and bisdemthoxycurcumin (**4e**, $IC_{50} = 4.0 \pm 1.2\ \mu M$).

Among these compounds, **4e**, in which two of the methoxy groups were removed from curcumin showed diminished inhibitory activity. Based on this result, we predicted that curcumin derivatives would have inhibitory activity against sialidase from *S. pneumoniae* Nan A and that the methoxy group played an important role in the inhibitory activity. Therefore, we modified curcumin and evaluated the inhibitory effects on *S. pneumoniae* Nan A (Table 1).

To evaluate the functionalisation of the curcumin skeleton to find a suitable backbone. Hydrogenation of the heptadienyl group (**6**, $IC_{50} = 82.1 \pm 7.6\,\mu M$) resulted in significantly diminished inhibitory activity. Next, to confirm the phenyl group at the 7-position, inhibitory activity after the addition of a pyridinyl (**5g**) or phenyl ring (**5h**) was compared. The results indicated that the pyridinyl group (**5 g,** $IC_{50} = 4.4 \pm 0.1\,\mu M$) was more effective than the phenyl group (**5 h**, $IC_{50} = 7.1 \pm 0.1\,\mu M$), but both compounds showed lower inhibitory activity than curcumin. To investigate the effect of 1,3-diketone, 4-hydroxy or 3-methoxy groups, synthesised and compared with six kinds of curcumins. First, to investigate the effect of the 1, 3-diketone moiety, pyrazole (**8**, $IC_{50} = 6.2 \pm 1.5\,\mu M$) or carbocyclic 1, 3-diketone (**4f**, $IC_{50} = 4.7 \pm 0.5\,\mu M$) was substituted. Second, to investigate the effect of the 4-hydroxyl groups in curcumin, acetyl (**7**, $IC_{50} = 2.6 \pm 0.6\,\mu M$) or methyl groups (**4c**, $IC_{50} = 3.4 \pm 1.0\,\mu M$) were substituted and conversion of the hydroxy at the *para* position to ester (**5i**, $IC_{50} = 1.0 \pm 0.5\,\mu M$). Third, to investigate the 3-methoxy groups, elimination of the 3-methoxy groups (**4d**, $IC_{50} = 1.5 \pm 0.7\,\mu M$) and conversion of the methoxy at the *meta* position to ethoxy (**5f**, $IC_{50} = 1.5 \pm 0.8\,\mu M$) were compared. Inhibitory activity of these compounds was diminished than curcumin (**4a**). This suggested that 1,3-diketone and a heptadienyl group were essential functional groups for sialidase inhibition and either a methoxy or a hydroxyl group was required.

Based on these results, we substituted a methoxy or hydroxyl group at the *para*- or *meta*- position of the 1,7-diphenylhepta-1,6-diene-3,5-dione backbone. To confirm the positional tendency of the methoxy and hydroxyl groups, we reacted it with feruloyl (**3a**) or isoferuloyl (**3b**) acetone or hispolon (**3c**) with the corresponding aldehyde **2**. Because a methoxy or hydroxyl group was

Table 1. Inhibitory effects of synthesised compounds against *S. pneumoniae* Nan A.

Compounds	IC_{50} (μM)[a]	Compounds	IC_{50} (μM)
4a	0.6 ± 0.1	**5i**	1.0 ± 0.5
4b	1.0 ± 0.4	**5j**	0.9 ± 0.3
4c	3.4 ± 1.0	**5k**	1.0 ± 0.1
4d	1.5 ± 0.7	**5l**	1.8 ± 0.2
4e	0.4 ± 1.2	**5m**	1.0 ± 0.2
4f	4.7 ± 0.5	**5n**	1.8 ± 0.4
5a	1.5 ± 0.7	**5o**	1.2 ± 0.1
5b	0.6 ± 0.1	**5p**	0.6 ± 0.2
5c	1.0 ± 0.4	**5q**	1.6 ± 0.1
5d	0.7 ± 0.2	**5r**	1.5 ± 0.2
5e	0.2 ± 0.1	**5s**	1.5 ± 0.1
5f	1.5 ± 0.8	**6**	82.1 ± 7.6
5g	4.4 ± 0.1	**7**	2.6 ± 0.6
5h	7.1 ± 0.1	**8**	6.2 ± 1.5

[a]IC_{50} values of compounds represent the concentration that caused 50% enzyme activity loss; all compounds were examined in a set of triplicates experiment.

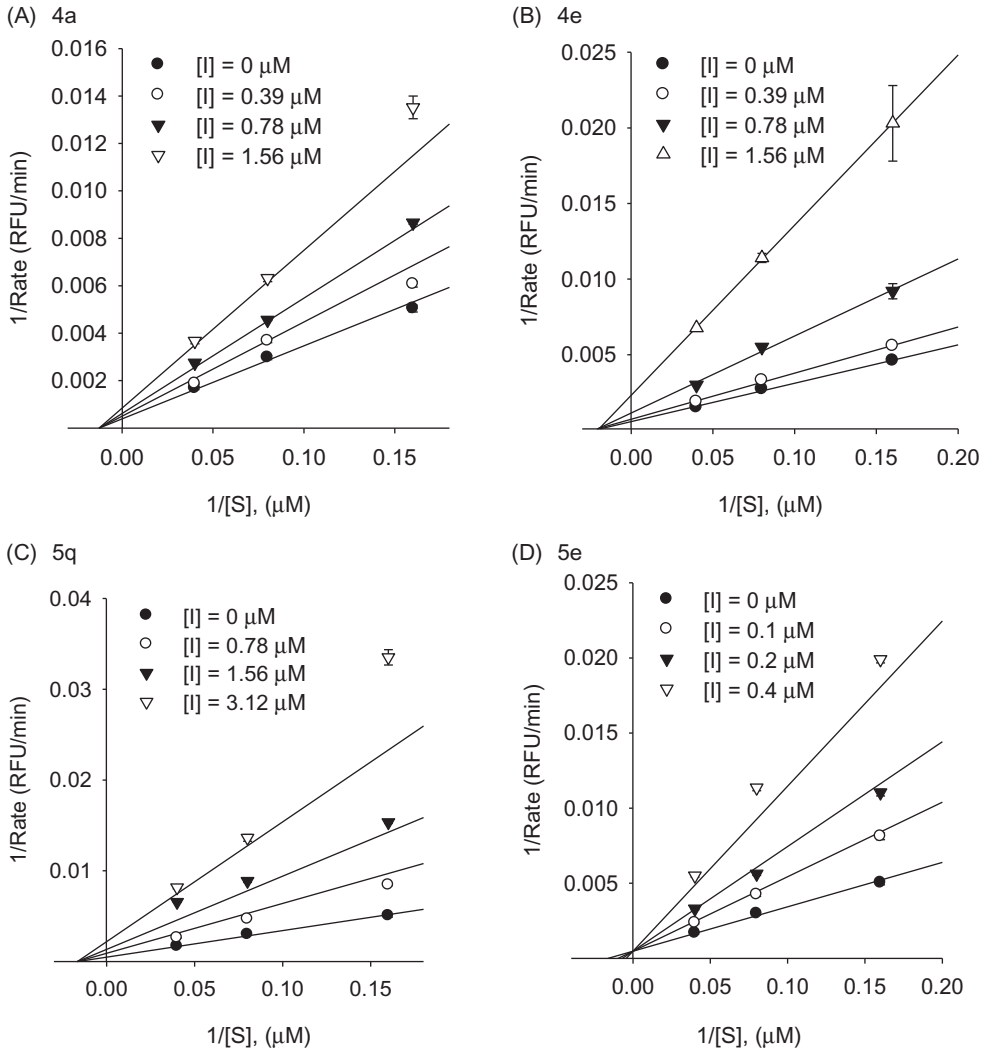


Figure 1. Graphical determination of the inhibition type for compounds **4a**, **4e**, **5q**, and **5e**. Lineweaver–Burk (A–D) plots for the inhibitory activity of compounds **4a**, **4e**, **5q**, and **5e**, respectively, against *S. pneumoniae* Nan A hydrolysis activity in the presence of different substrate concentrations.

Table 2. Inhibitory effects of curcumin derivatives in *V. cholera* and *C. perfringens* sialidase.

	IC_{50} (μM)[a]			IC_{50} (μM)	
Compound	*V. cholerae*	*C. perfringens*	Compound	*V. cholerae*	*C. perfringens*
4a	5.3 ± 0.7	1.6 ± 0.4	**5g**	43.6 ± 0.1	32.9 ± 5.1
4b	7.6 ± 0.5	3.2 ± 0.4	**5i**	9.5 ± 1.5	3.3 ± 0.4
4c	21.5 ± 7.0	13.1 ± 2.2	**5j**	6.9 ± 1.8	1.6 ± 0.5
4d	11.9 ± 2.0	6.1 ± 0.8	**5n**	6.8 ± 0.8	5.9 ± 0.8
4e	15.1 ± 0.1	11.0 ± 0.8	**5o**	4.2 ± 0.8	2.8 ± 0.3
4f	24.9 ± 1.8	13.4 ± 0.1	**5p**	4.3 ± 0.7	2.1 ± 0.4
5a	11.7 ± 0.2	6.7 ± 0.5	**5q**	2.9 ± 0.9	1.7 ± 0.1
5b	9.2 ± 0.2	6.7 ± 0.8	**5r**	1.9 ± 0.2	1.3 ± 0.2
5c	7.7 ± 0.7	3.5 ± 0.2	**6**	NA[b]	269.4 ± 31.2
5d	2.7 ± 0.8	1.0 ± 0.1	**7**	37.5 ± 3.5	13.8 ± 0.6
5e	4.8 ± 1.5	0.5 ± 0.07	**8**	41.6 ± 18.7	9.5 ± 0.1
5f	6.7 ± 0.6	2.9 ± 0.5			

[a]IC_{50} values of compounds represent the concentration that caused 50% enzyme activity loss; all compounds were examined in a set of triplicates experiment.
[b]No activity.

essential for sialidase inhibition, compounds **4c** and **4d** were excluded from consideration. As shown in Table 1, IC_{50} values ranged from 0.2–1.5 μM, indicating there was no positional tendency for inhibition (**4a**, **4 b,** and **5a–5e** in Table 1). Among the examined compounds, **5e**, containing the catechol with the isoferuloyl moiety, was the most potent inhibitor ($IC_{50} = 0.2 \pm 0.1$ μM), with a 3.0-fold improvement in inhibitory activity over that of curcumin. Thereafter, to investigate the electronic effect, we substituted an electron-donating group (EDG) or electron-withdrawing group (EWG) into the *para* position of the phenyl rings, including nitro (**5j**), trifluoromethyl (**5k**), fluoro (**5l**), bromo (**5m**), acetamido (**5n**), methoxy (**5o**), hydroxyl (**5p**), amino (**5q**), *N*, *N*-dimethylamino (**5r**), and piperidinyl (**5s**) groups. The IC_{50} values ranged from 0.6–1.8 μM and the electronic effect did not influence in Nan A inhibition. Based on these observations, we investigated the kinetic mechanisms of inhibitors with IC_{50} values of 25 μM or less. We selected the major components of *Turmeric* (**4a**, **4e**, **5q**) and compound **5e** as the most potent inhibitors for the kinetic study. We found that the major components of *Turmeric* (**4a**, **4e**, **5q**) showed noncompetitive inhibition characteristics with a K_i of 1.3 μM (**4a**), 1.2 μM (**4e**), and 0.8 μM (**5q**), respectively. Conversely, compound **5e** exhibited a potent competitive inhibition against Nan A with a K_i of 0.14 μM (Figure 1).

Synthesised compounds were then evaluated for their inhibitory effect on sialidase from *Vibrio cholerae* and *Clostridium perfringens*, which also release sialidase and play a role in the pathogenesis. The inhibitory assay results are summerised in Table 2.

Similar to the above results, methoxy or hydroxyl, heptadienyl and α, β-unsaturated ketone groups played important roles in the inhibitory activity. Although the position of the methoxy and hydroxyl group did not influence the inhibition of the above enzymes, the inhibitory effect increased with the greater substitution of hydroxyl groups in the phenyl rings. Thereafter, we evaluated inhibitor activity to confirm the electronic effect. *C. perfringens* sialidase inhibitory activity was not affected by the electronic effect. Among the examined compounds, **5e** ($IC_{50} = 0.5 \pm 0.07$ μM), containing the catechol moiety, was the most potent inhibitor of *C. perfringens* and displayed a 3.2-fold improvement over curcumin. Conversely, substitution of an electron donating groups at the *para* position of the phenyl group resulted in better potency than the substitution of an electron withdrawing groups against *V. cholera* sialidase. Among the examined compounds, compound **5r** (IC_{50}=1.9 ± 0.2 μM), containing the *N*, *N*-dimethylamino group, was the most potent and displayed a 2.7-fold improvement in inhibitory activity over that of curcumin. The IC_{50} values for sialidase inhibitory activity were 2.7–43.6 μM in *V. Choleara* and 0.5–269.4 μM in *C. perfringens*.

4. Conclusions

In conclusion, we developed, for the first time, inhibitors of sialidase from *S. pneumoniae* Nan A, *V. cholerae* and *C. perfringens* using curcumin derivatives. Of the 28 compounds synthesised, 7-(3,4-dihydroxyphenyl)-5-hydroxy-1-(3-hydroxy-4-methoxyphenyl)hepta-1,4,6-trien-3-one (**5e**), which is novel compound contained isoferuloyl and catechol moieties, was the most potent inhibitors of *S. pneumoniae* ($IC_{50} = 0.2 \pm 0.1$ μM) and *C. perfringens* ($IC_{50} = 0.5 \pm 0.07$ μM). All major *Turmeric* components showed noncompetitive inhibition, but compound **5e** exhibited competitive inhibition against *S. pneumoniae* Nan A. In the case of *V. cholerae* sialidase, 7-(4-(dimethylamino)-phenyl)-5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)hepta-1,4,6-trien-3-one (**5r**), containing *N*, *N*-dimethylamino as an electron-donating group, was the most potent inhibitor ($IC_{50} = 1.9 \pm 0.2$ μM). The SAR analysis suggested that the α, β-unsaturated ketone, heptadienyl and either the methoxy or hydroxy group in curcumin was required for sialidase inhibition. These results indicated that curcumin analogues may potentially be used for sepsis caused by bacterial sialidase. Further *in vivo* evaluation of compound **5e** and **5r** will be performed in our laboratory.

Disclosure statement

No potential conflict of interest was reported by the authors.

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