Antibacterial and antioxidant activity evaluation of novel symmetrical and unsymmetrical C5-curcuminoids

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Curcumin remains one of the most widely studied natural product due to its wide range of biological activities but because of the presence of central β-diketone unit which is responsible for its poor bioavailability, this molecule cannot be developed as a drug. In order to overcome this, curcumin has been modified to metabolically stable symmetrical and unsymmetrical C5-curcuminoids and their in vitro antibacterial and antioxidant activity were studied. Few of the synthesized C5-curcuminoids (10, 11 and 25) displayed excellent potency (MIC value 1.5 to 6.25 µg/mL) against the tested bacterial strains. Six of the analogues (10, 12-15 and 25) were also found to exhibit good antioxidant activity (IC50 values 33.87 to 49.45 µg/mL) in a DPPH free radical scavenging assay. The test compounds have been further subjected to in silico ADMET analysis and various pharmacokinetic properties were calculated. Compounds 20, 23-25 are predicted to have less toxic effects and follow the permissible pharmacokinetic criterion.

Keywords: Curcumin, C5-curcuminoids, antibacterial, antioxidant

Curcumin (Figure 1a), a principle constituent of the perennial herb Curcuma longa, is well known for its medicinal potential since 1900 BC. Its use in liver injury, arthritis, cataract formation, cardiovascular diseases, Alzheimer, diabetes and wound healing has been well documented in Indian Ayurveda1,2. Due to its ability to interact with multiple molecular signaling pathways involved in carcinogenesis, it has been the subject of intense pharmacological studies globally in both industry and academia3-5. Curcumin is also known to exhibit potent antibacterial activity against both gram-negative and gram-positive bacteria including several multi-drug resistant bacterial strains6-11. In addition, being a free radical scavenger, it was found to be an effective antioxidant in different in vitro assays including DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] and DMPD (N,N-dimethyl- p-phenylenediamine dihydrochloride) radical scavenging, hydrogen peroxide scavenging and metal chelating activities12. The antioxidant properties of curcumin plays a significant role in inhibiting peroxidation of membrane lipids and oxidative damage of DNA and proteins associated with various pathological conditions such as atherosclerosis and neurodegenerative diseases13.

Curcumin’s excellent medicinal potency was attributed to its safe toxicity profile and in a phase I clinical trial it was demonstrated that curcumin is safe and non-toxic even at high oral doses of 12 g/day14. In spite of these brilliant pharmaceutical properties, curcumin suffers from several disadvantages like poor systemic bioavailability, rapid metabolism and low solubility which in turn reduces its capability of becoming a potential drug candidate15. The presence of central β-diketone functionality in curcumin was found to be the main culprit behind these observations, which is a substrate for liver aldotoketo reductases and hence may contribute for the rapid metabolism of curcumin in vivo16. However, modification of central β-diketone moiety of curcumin to mono-keto group leads to the development of C5-curcuminoids having 1,5-diaryl-3-oxo-1,4-pentadienyl pharmacophore. The C5-curcuminoids have shown better pharmacological profile like superior bioavailability, high potency and improved metabolic stability when compared with curcumin and other C7-curcuminoids17-22. Encouraged by the safety profile and potent activity of C5-curcuminoids, we recently reported cytotoxic potential of some symmetrical C5-curcuminoids17,23,24. In continu-
to this study, herein is reported the synthesis of symmetrical and unsymmetrical C5-curcuminoids and evaluation of their antibacterial and antioxidant activities.

Results and Discussion

Chemistry

The chemistry for the synthesis of symmetrical C5-curcuminoids is straightforward and is similar to the synthesis reported in our previous publication. In short, 3-bromo-4-hydroxy-5-methoxybenzaldehyde was treated with an excess of linear aliphatic chain dibromoalkanes with C-2 or C-3 carbon spacer in the presence of a base to get corresponding benzaldehydes with free bromo group at terminal position (Scheme I). These benzaldehydes were then treated with an excess of different amino functionalities to yield the corresponding amino substituted aldehydes.
4-9 in excellent yields. Finally, the resulting benzaldehydes 4-9 were subjected to Claisen-Schmidt condensation in the presence of cyclopentanone to yield the desired C5-curcuminoids 12-17. The synthesis of C5-curcuminoids 10 and 11 were achieved when substituted benzaldehydes 2 and 3 were subjected to Claisen-Schmidt condensation with cyclopentanone.

For the synthesis of unsymmetrical C5-curcuminoids, commercially available 1-napthaldehyde 18 was used and reacted with an excess of acetone to get intermediate 19 (Scheme II). The intermediate 19 was then subjected to a Claisen-Schmidt type of condensation with different substituted aldehydes to get the unsymmetrical C5-curcuminoids 21-26 in good to excellent yield. Synthesis of C5-curcuminoid containing anthracene moiety 20 was achieved by reacting intermediate 19 with 9-anthraldehyde in the presence of NaOH as a base.

**In vitro Antibacterial Activity**

The antibacterial activity of all the synthesized symmetrical 10-17 and unsymmetrical C5-curcuminoids 20-26 were determined against three gram negative bacterial strains viz. *Xanthomonas oryzae*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* and one gram positive bacterial strain viz. *Staphylococcus aureus* in a disk diffusion assay using Streptomycin as a standard antibiotic. The minimum inhibitory concentration (MIC) of compounds which shows good to very good activity in disk diffusion assay was determined based on serial dilution method. In the symmetrical C5-curcuminoids series 10-17, two of the compounds (10 and 11) showed better activity with lower minimum inhibitory concentration (MIC) values against *S. aureus* and *P. aeruginosa* strains when compared with Streptomycin (Table I). However, when the bromo group of these compounds (10 and 11) was substituted with amino functionalities 12-17, activity dropped significantly. It was also found that when the alkyl chain length in these analogues was increased from C2 to C3, the activity pattern doesn’t alter much. This gives an insight that the length of the spacer doesn’t have a great impact on activity profile of these compounds. In unsymmetrical C5-curcuminoid series 20-26, compounds with fluoro and chloro group were inactive (21 and 22) while bromo containing compound 23 showed improved activity against *K. pneumonia*, *S. aureus* and *P. aeruginosa*. Incorporation of two chloro group in the phenyl moiety (24 and 25) improved activity. But, when instead of two chloro groups, two fluoro groups are introduced 26 the activity dropped against all the bacterial strains. Three compounds 10, 11 and 25 showed better activity against *S. aureus* and *P. aeruginosa* when compared with streptomycin.

It would be noted that except compound 15, 21 and 26 which were not active against any of the tested bacteria, rest of compounds have shown activity against at least one of the tested bacteria with MIC in range of 1.562-12.5 µg/mL. Further, except compounds 12, 15, 21, 22 and 26, rest of the compounds were found to be active against both gram positive and gram negative bacteria which could be helpful in generating next generation broad spectrum antibiotics through systematic chemical modifications and optimizations.

**In vitro Antioxidant Activity**

In vitro antioxidant activity of all the synthesized compounds was determined by DPPH free radical scavenging activity using gallic acid as a reference compound (Table II). This method is based on measuring the continual absorbance decrease of the methanolic solution of the DPPH at 517 nm, in the

![Scheme II](image-url)

Scheme II — (a) Acetone, 20% KOH (w/v), EtOH, RT, 4h, 75-85% (b) Substituted benzaldehydes, 20% NaOH (w/v), EtOH, RT, 4h, 80-90%; (c) 9-Anthraldehyde, 20% NaOH (w/v), EtOH, RT, 4h, 80-90%.
Table I — Antibacterial activity of synthesized symmetrical and unsymmetrical C5-curcuminoids

<table>
<thead>
<tr>
<th>Compd</th>
<th>n</th>
<th>R</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Gram Negative Bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>* Xanthomonas oryzae</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
<td>Br</td>
<td>12.5</td>
</tr>
<tr>
<td>11</td>
<td>2</td>
<td>Br</td>
<td>6.25</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>Piperidine</td>
<td>*</td>
</tr>
<tr>
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<td>1</td>
<td>2-methyl piperidine</td>
<td>12.5</td>
</tr>
<tr>
<td>14</td>
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<td>2</td>
<td>Piperidine</td>
<td>*</td>
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<td>2-methyl piperidine</td>
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<td>17</td>
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<td>Morpholine</td>
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<td>20</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>4-F</td>
<td>*</td>
</tr>
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<td>22</td>
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<tr>
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<td>2,4-di-Cl</td>
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<td>-</td>
<td>2,6-di-Cl</td>
<td>*</td>
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<tr>
<td>26</td>
<td>-</td>
<td>3,5-di-F</td>
<td>*</td>
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<tr>
<td>Streptomycin</td>
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* = Not active up to 5000 (µg/mL)

Table II — IC₅₀ values of synthesized C5-curcuminoids using DPPH assay

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<thead>
<tr>
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<th>IC₅₀ (µg/mL)</th>
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<td>Gallic Acid</td>
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Table III — Prediction of Lipinski’s ‘Rule of 5’ for active anti-bacterial and anti-oxidant compounds

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<tr>
<th>Compd</th>
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<th>Donor HB</th>
<th>Accept HB</th>
<th>QPlogPo/w</th>
<th>Rule Of Five</th>
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<td>6.063</td>
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<tr>
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<td>7</td>
<td>3.037</td>
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<td>-5.975</td>
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<td>4</td>
<td>-0.585</td>
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*All values calculated by QikProp v 3.527 and the explanations of the descriptors are given in the text

As a primary test of the drug-likeness of the compounds, we calculated Lipinski’s rule of 5 using QikProp. According to Lipinski’s rule of 5, compounds should not have more than 5 hydrogen bond donors (donorHB), 10 hydrogen bond acceptors (acceptHB), molecular weights (mol_MW) less than 500 amu, and partition coefficients between octanol and water (QPlogP (oct/wat)) less than 5. The Qikprop results as depicted in Table III shows various parameters of Lipinski’s rule of 5 calculated for the test compounds. An orally active compound should not have more than four violations of these rules29. In the present study, active compounds 10-13 and 15 had two violations of Lipinski rule (with MW > 500 and (QPlogP (oct/wat) > 5) whereas compounds 14 (MW > 500) and 20-25 with (QPlogP (oct/wat) > 5) showed one violation of Lipinski’s rule. Prediction of oral drug absorption (Percent Human Oral Absorption) of test compounds show satisfactory results which are comparable to reference compound curcumin, with the exception for compounds 12 and 14 showing moderate values (Table IV). The discrepancy in prediction of oral bioavailability was observed for the reference compounds streptomycin and gallic acid. According to several reports, the oral bioavailability is influenced by compound’s flexibility and are thus measured by the number of rotatable bonds (<15) and polar surface area (70 Å²-200 Å²). Use of descriptors, polar surface area and rotatable bonds for predicting bioavailability has to be considered with caution with respect to choice of descriptor algorithm used and can be influenced by several other factors29-31. However, a better approach

**In silico Evaluation of Pharmacokinetic Properties**

The pharmacokinetic properties of the compounds showing good antibacterial and antioxidant properties were predicted using ADMET descriptors in Qikprop27 and Discovery Studio28 programs. Some of the important ADMET parameters calculated and its permissible ranges are listed in Table III and IV.
is by considering a total sum of H-bond donors and acceptors criterion ($\leq 12$), an algorithm independent descriptor along with polar surface area criterion. In the present study, all the test compounds have a number of rotatable bonds <15 and polar surface area for gallic acid, the standard reference compound for anti-oxidant activity, showed values in permissible ranges. Further, the results of Caco-2 cells permeability (QPPCaco), a model used for the gut–blood barrier, for compounds 12-15 showed intermediate values. The reference compounds streptomycin and gallic acid show poor QPPCaco prediction of 0.076 and 10 respectively. The aminoglycosides such as streptomycin are known to have poor oral absorption and get poorly absorbed from the gastrointestinal tract for being highly polar cations. However, in contrast to the descriptor prediction for intestinal permeability by Qikprop, it is reported that gallic acid get readily absorbed from the gastrointestinal tract. The problem in the accuracy can be due to the discrepancy in QPPCaco prediction by Qikprop, which are performed for specific conditions ($pH$ 7) and get influenced by conditions such as active vs passive transport mechanisms, efflux pump mechanisms, different experimental conditions than that used in the regression analysis, etc. Moreover, the ADMET_Absorption_Level calculations by Discovery Studio have correctly predicted a good intestinal absorption property for gallic acid (Table IV).

Furthermore, the prediction for human serum albumin binding (QPlogKhsa,) shows all inhibitors, predicted to lie within the expected range for 95% of known drugs (-1.5 to 1.5). Also, the QikProp descriptor for brain/blood partition coefficient (QPlogBB) and the blood-brain barrier mimic MDCK cell permeability (QPPMDCK) show satisfactory predictions for all the test compounds and the reference compound gallic acid. The exception being streptomycin, which is reported to get largely excluded from most cells and from the central nervous system.

Experimental Section

All the chemicals used in the synthesis were purchased from Sigma-Aldrich (India) and were used as received. Thin layer chromatography was employed to monitor the progress of the reactions and checked using precoated TLC plates (E. Merck Kieselgel 60 F$_{254}$, Merck KGaA, Darmstadt, Germany) with spots being visualized by iodine vapors. Compounds were purified by precipitation or recrystallization technique with suitable solvents. Solvents were distilled before use for purification purposes. Melting points were recorded on an ERS automated melting point apparatus (Stanford Research System, California) and are uncorrected. IR spectra were recorded using Perkin-Elmer spectrophotometer (U.S.A) and the values are expressed as $\text{cm}^{-1}$. Mass spectral data were recorded on a Jeol-AccuTOF JMS-T100LC (Japan) and micromass LCT Mass Spectrometer/Data system. Elemental analyses were performed on Carlo Erba Model 1106.

![Table IV — Calculated ADMET properties](image_url)

<table>
<thead>
<tr>
<th>Compd</th>
<th>a$^*$</th>
<th>b$^*$</th>
<th>c$^*$</th>
<th>d$^*$</th>
<th>e$^*$</th>
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a: Percent Human Oral Absorption (>80%-high, <25%-poor); b: QPPCaco nms (<25-poor, >500-great); c: ADMET Absorption Level (0-high, 1-moderate, 2-low, 3-very low); d: QPlogBB (-3.0-1.2); e: QPMDCK (<25-poor, >500-great); f: QPlogKhsa (-1.5 to 1.5); g: PSA (7.0-200.0); h: rotor (0-15).

*Calculated using QikProp v 3.5. Range/recommended values calculated for 95% known drugs.

#Calculated using Discovery Studio v 2.5. Calculated from logP and polar surface area a set of 199 passively well-absorbed molecules.
EA-1108 elemental analyser and data of C, H and N is within ± 0.4 of calculated values. 1H and 13C NMR spectra were recorded on Jeol Spectrospin spectrometer (Japan) at 400 MHz and 100 MHz respectively using TMS as an internal standard. The chemical shift values are recorded on δ scale and the coupling constants (J) are in Hz.

General procedure for the synthesis of substituted benzaldehydes, 2-3

To a solution of 3-bromo-4-hydroxy-5-methoxybenzaldehyde 1 (1 mmol) and anhydrous K$_2$CO$_3$ (3 mmol) in 20 mL DMF, requisite linear chain aliphatic dibromoal-kanes (5 mmol) was added. The reaction mixture was then stirred at 80°C for 1 h, cooled to 0°C on an ice bath, quenched with 1N HCl and then extracted with CHCl$_3$. The organic layer was washed with water and dried over anhydrous Na$_2$SO$_4$. Excess solvent was evaporated under reduced pressure to obtain oily residue of corresponding amino substituted aldehydes 4-9, which were used as such for further reaction.

General procedure for the synthesis of symmetrical C5-curcuminooids, 10-11

To a solution of substituted benzaldehydes 2,3 (15 mmol) in MeOH (5 mL), cyclopentanone (7.5 mmol) was added. The solution was allowed to stir for 15 min at RT, followed by dropwise addition of 20% (w/v) aq. NaOH solution. The reaction mixture was stirred at RT and progress of reaction was monitored by TLC. After completion of reaction, saturated solution of NH$_4$Cl was added to the reaction mixture. The precipitate thus formed was washed successively with water, cold methanol and finally with cold acetone. It was then dried and purified by recrystallization from ethanol/water system to obtain corresponding curcumin derivatives 10-11.

General procedure for the synthesis of symmetrical C5-curcuminooids, 12-17

To a solution of amino substituted benzaldehydes 4-9 (15 mmol) in MeOH (5 mL), cyclopentanone (7.5 mmol) was added. The solution was allowed to stir for 15
min at RT, followed by dropwise addition of 20% (w/v) aq. NaOH solution. The reaction mixture was stirred at RT and progress of reaction was monitored by TLC. After completion of reaction, saturated solution of NH₄Cl was added to the reaction mixture. The precipitate formed was washed successively with water, cold methanol and finally with cold acetone. It was then dried and purified by recrystallization from ethanol/water system to obtain corresponding curcumin derivatives 12-17.

(2E,5E)-2,5-Bis(3-bromo-5-methoxy-4-(2-(piperidin-1-yl)ethoxy)benzylidene) cyclopentanone, 12: Yield 90%; m.p. 184-187°C; IR (Film): 2933, 1618 (C=O), 1490, 1322, 1236, 1047 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.42-1.47 (m, 4H, piperidine ring), 1.57-1.63 (m, 8H, piperidine ring), 2.87-2.97 (m, 8H, 4 × NCH₂), 3.58-3.62 (t, 4H, J = 5.8 Hz, 2 × NCH₂), 3.77-3.81 (m, 8H, morpholine), 4.18 (t, 4H, J = 5.8 Hz, 2 × OCH₂), 7.02 (d, 2H, J = 1.6 Hz, 2 × ArH), 7.40 (d, 2H, J = 1.6 Hz, 2 × ArH), 7.44 (s, 2H, 2 × CH=CH-C=C=O); ESI-MS: m/z 733.21 (M+H)⁺, 733.20 (M+2)⁺. Anal. Calc'd for C₃₅H₃₈Br₁N₀₁O₂: C, 57.39; H, 6.05; N, 3.07. Found: C, 57.41; H, 6.15; N, 3.81%.

(2E,5E)-2,5-Bis(3-bromo-5-methoxy-4-(2-(2-methylpiperidin-1-yl)ethoxy)benzylidene) cyclopentanone, 13: Yield 75%; m.p. 178-181°C; IR (Film): 2926, 1617 (C=O), 1489, 1320, 1283, 1238, 1047 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.12 (d, 6H, J = 8 Hz, 2 × CH₃), 1.28-1.32 (m, 4H, methylpiperidinering), 1.55-1.67 (m, 8H, methylpiperidinering), 2.36-2.45 (m, 4H, 2 × NCH₂), 2.82-2.89 (m, 4H, 2 × NCH₂), 2.92-2.98 (m, 2H, 2 × NCH), 3.10 (s, 4H, 2 × CH₂N=C=), 3.89 (s, 6H, 2 × OCH₃), 4.15 (t, 4H, J = 5.8 Hz, 2 × OCH₂), 7.04 (d, 2H, J = 1.6 Hz, 2 × ArH), 7.40 (d, 2H, J = 1.6 Hz, 2 × ArH), 7.45 (s, 2H, 2 × CH=CH-C=C=O); ESI-MS: m/z 759.25 (M+H)⁺, 761.25 (M+2)⁺. Anal. Calc'd for C₃₇H₃₉Br₁N₁O₂: C, 58.43; H, 6.36; N, 3.68. Found: C, 58.56; H, 6.41; N, 3.75%.

(2E,5E)-2,5-Bis(3-bromo-5-methoxy-4-(3-(2-methylpiperidin-1-yl)propoxy)benzylidene) cyclopentanone, 15: Yield 88%; m.p. 163-166°C; IR (Film): 2929, 1618 (C=O), 1491, 1323, 1326, 1201, 1047 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 1.42-1.46 (m, 4H, piperidine ring), 1.58-1.60 (m, 8H, piperidine ring), 1.98-2.03 (m, 4H, 2 × CH₂), 2.40-2.44 (m, 8H, piperidine ring), 2.55 (t, 4H, J = 5.8 Hz, 2 × NCH₂), 3.10 (s, 4H, 2 × CH₂CH₂C=), 3.88 (s, 6H, 2 × OCH₃), 4.10 (t, 4H, J = 5.8 Hz, 2 × OCH₂), 7.03 (d, 2H, J = 1.6 Hz, 2 × ArH), 7.40 (d, 2H, J = 1.6 Hz, 2 × ArH), 7.44 (s, 2H, 2 × CH=CH-C=C=O); ESI-MS: m/z 759.24 (M+H)⁺, 761.23 (M+2)⁺. Anal. Calc'd for C₃₉H₃₈Br₁N₁O₂: C, 58.43; H, 6.36; N, 3.68. Found: C, 58.56; H, 6.41; N, 3.75%.

Procedure for the synthesis of (E-4)-(naphthalen-1-yl)but-3-en-2-one 19 (Ref 36)

To a solution of acetone (4 mmol) in EtOH (5 mL), aqueous KOH (20% w/v, 1 mL) was added and stirred for 20 min at 0-5°C. Then 1-naphthaldehyde 18 (1 mmol) was added drop-wise and the reaction mixture was allowed to stir at RT until complete disappearance of
aldehyde was observed. After completion of reaction which took around 4h, the reaction mixture was quenched with saturated solution of NH₄Cl (20 mL) and extracted with chloroform (2 × 30 mL). The combined organic phase was dried over anhydrous Na₂SO₄, and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel to provide yellow oil of compound 19. Yield 85%; ¹H NMR (400 MHz, CDCl₃): δ 2.47 (s, 3H, CH₃), 6.82 (d, 1H, J = 15.3 Hz, vinyl CH), 7.48-7.61 (m, 3H, ArH), 7.78 (d, 1H, J = 8.7 Hz, ArH), 7.88-7.92 (m, 2H, ArH), 8.18 (d, 1H, J = 8.7 Hz, ArH), 8.37 (d, 1H, J = 16.1 Hz, vinyl CH); ESI-MS: m/z 197.13 (M+H)⁺. Anal. Calcd for C₁₃H₁₂O: C, 85.68%; H, 6.16%. Found: C, 85.71%; H, 6.15%.

General procedure for the synthesis of unsymmetrical C₅-curcuminoids, 20-26

Compound 19 (0.75 mmol) and substituted benzaldehydes (0.75 mmol) were dissolved in EtOH (5 mL) at RT. To this, 2 mL of aqueous NaOH (20% w/v) was added and stirred for 4h. After completion of reaction as evident by TLC, saturated solution of NH₄Cl was added to the reaction mixture. The precipitate formed was filtered, washed with water and then with cold ethanol. It was then dried and purified by recrystallization from ethanol to obtain corresponding compounds 20-26.

(1E, 4E)-1-(4-Chlorophenyl)-5-(naphthalen-1-yl)-penta-1,4-dien-3-one, 22: Yield 78%; m.p. 130-132°C; IR (Film): 3019, 2923, 2855, 1653, 1615 (C=O), 1491, 1345, 1213, 1086, 980, 753, 669 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.08 (d, 1H, J = 16.1 Hz, vinyl CH), 7.16 (d, 1H, J = 15.3 Hz, vinyl CH), 7.39-7.41 (m, 2H, ArH), 7.52-7.60 (m, 5H, ArH), 7.72 (d, 1H, J = 15.3 Hz, vinyl CH), 7.78-7.93 (m, 3H, ArH), 8.25 (d, 1H, J = 8.7 Hz, ArH), 8.59 (d, 1H, J = 16.1 Hz, vinyl CH); ¹³C NMR (100 MHz, CDCl₃): δ 123.39, 125.13, 125.45, 126.12, 126.31, 126.98, 127.65, 128.79, 129.26, 129.54, 130.87, 131.69, 132.10, 133.26, 133.72, 136.43, 140.47 (vinyl C), 142.00 (vinyl C), 188.48 (C=O); ESI-MS: m/z 319.13 (M+H)⁺, 321.13 (M+2)⁺. Anal. Calcd for C₂₁H₁₅ClO: C, 79.12%; H, 4.74%. Found: C, 79.17%; H, 4.78%.

(1E,4E)-1-(4-Bromophenyl)-5-(naphthalen-1-yl)-penta-1,4-dien-3-one, 23: Yield 85%; m.p. 148-150°C; IR (Film): 3051, 2922, 2853, 1652, 1615 (C=O), 1486, 1399, 1345, 1188, 1110, 1071, 981, 808, 777 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.11 (d, 1H, J = 16.1 Hz, vinyl CH), 7.17 (d, 1H, J = 16.1, vinyl CH), 7.47-7.61 (m, 7H, ArH), 7.71 (d, 1H, J = 16.1 Hz, vinyl CH), 7.85-7.93 (m, 3H, ArH), 8.25 (d, 1H, J = 8.7 Hz, ArH), 8.59 (d, 1H, J = 16.1 Hz, vinyl CH); ¹³C NMR (100 MHz, CDCl₃): δ 123.39, 124.82, 125.13, 125.45, 126.12, 126.31, 126.98, 127.65, 128.79, 129.26, 129.54, 130.87, 131.69, 132.10, 133.26, 133.72, 136.43, 140.47 (vinyl C), 188.48 (C=O); ESI-MS: m/z 363.09 (M+H)⁺, 365.08 (M+2)⁺. Anal. Calcd for C₂₁H₁₅BrO: C, 69.44%; H, 4.16%. Found: C, 69.49%; H, 4.18%.

(1E,4E)-1-(2, 6-Dichlorophenyl)-5-(naphthalen-1-yl)-penta-1,4-dien-3-one, 24: Yield 80%; m.p. 148-150°C; IR (Film): 3058, 2924, 2854, 1656, 1612 (C=O), 1468, 1346, 1252, 1188, 1102, 980, 869, 779 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 7.05 (d, 1H, J = 16.1 Hz, vinyl CH), 7.20 (d, 1H, J = 15.3 Hz, vinyl CH), 7.30-7.32 (m, 1H, ArH), 7.47-7.48 (m, 1H, ArH), 7.50-7.62 (m, 3H, ArH), 7.67 (d, 1H, J = 8 Hz, ArH), 7.86-7.96 (m, 3H, ArH), 8.09 (d, 1H, J = 16.1 Hz, vinyl CH), 8.25 (d, 1H, J = 8 Hz, ArH), 8.60 (d, 1H, J = 15.3 Hz, vinyl CH); ¹³C NMR (100 MHz, CDCl₃): δ 123.34, 125.15, 125.45, 126.32, 127.00, 127.11, 127.60, 128.40, 128.60, 128.80, 130.08, 130.96, 131.65, 131.68, 131.98, 133.71, 135.93, 136.49, 137.84 (vinyl C), 140.84 (vinyl C), 188.41 (C=O); ESI-MS: m/z 353.10 (M+H)⁺, 355.09 (M+2)⁺. Anal. Calcd for C₂₃H₁₅ClO: C, 71.40%; H, 3.99. Found: C, 71.45%; H, 3.96%.

(1E,4E)-1-(2, 6-Dichlorophenyl)-5-(naphthalen-1-yl)-penta-1,4-dien-3-one, 25: Yield 76%; m.p. 118-120°C; IR (Film): 3051, 2923, 2854, 1658, 1616 (C=O),
1510, 1313, 1258, 1185, 1108, 978, 877, 775, 656 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 717 (d, 1H, J = 15.3 Hz, vinyl CH), 7.22 (d, 1H, J = 8 Hz, ArH), 7.26-7.3 (m, 1H, vinyl CH), 7.40 (d, 2H, J = 8 Hz, ArH), 7.49-7.63 (m, 3H, ArH), 7.82-7.95 (m, 4H, 3 × ArH, vinyl CH), 8.25 (d, 1H, J = 8 Hz, ArH), 8.61 (d, 1H, J = 15.3 Hz, vinyl CH); ESI-MS: m/z 353.09 (M+H)+, 355.09 (M+2)+. Anal. Calcd for C₁₅H₁₄Cl₂O: C, 71.44; H, 3.40%. Found: C, 71.44; H, 3.98%.

(1E,4E)-1-(3,5-Difluorophenyl)-5-(naphthalen-1-yl)penta-1,4-dien-3-one, 26: Yield 75%; m.p. 126-128°C; IR (Film): 3055, 2922, 2854, 1667, 1622 (C=O), 1588, 1244 cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 6.83-6.90 (m, 1H, ArH), 7.08-7.18 (m, 4H, vinyl CH, 3 × ArH), 7.51-7.68 (m, 4H, 3 × ArH, vinyl CH), 7.86-7.95 (m, 3H, 2 × ArH, vinyl CH), 8.26 (d, 1H, J = 8 Hz, ArH), 8.61 (d, 1H, J = 15.3 Hz, vinyl CH); ESI-MS: m/z 321.16 (M+H)+. Anal. Calcd for C₁₅H₁₄F₂O: C, 78.74; H, 4.41. Found: C, 78.77; H, 4.40%.

Materials and methods for biological activity determination

*Xanthomonas oryzae* (ITCC B-47) was procured from Indian Type Culture Collection (ITCC) center, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012, India and *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 2581) and *Klebsiella pneumoniae* (MTCC 7028) were procured from the microbial type culture collection and gene bank, Institute of Microbial Technology, Chandigarh. Gallic acid and 1,1-di-phenyl-2-picrylhydrazyl (DPPH) was procured from Sigma Aldrich. Glacial acetic acid, hydrochloric acid, hexane, methanol, tris buffer and sodium acetate were procured from Merck India Ltd., and ready-made Nutrient agar and broth was purchased from Hi-Media Lab, New Delhi.

**Disk diffusion assay for determining antibacterial activity**

The antibacterial potential of synthesized compounds was tested using a disk diffusion assay. Briefly, the nutrient agar medium (25 mL) was poured into petri dishes (90 cm in diameter) under aseptic conditions in a laminar flow hood. The plates were kept in the laminar flow chamber for solidification of the media. After solidification, 100 µL of fresh culture (log phase) was spread on the surface of the solidified medium with the help of a spreader. The plates were then kept in laminar flow for drying. Once dried, five plain sterile dishes were placed in the plate and 5 µL of test solution of different concentration was loaded on each disk. In control plate, commercially procured Streptomycin (25 µg/disk) was used. Plates were then kept at 37°C. After 24 h, plates were taken out from the incubator and zone of inhibition (in mm) was recorded for all the compounds tested and commercial antibiotic. All experiments were in triplicate for each treatment against each bacteria. The minimum inhibitory concentration (MIC), defined as the lowest concentration of material that inhibits the growth of an organism was determined based on serial dilution method, varying concentration of test compounds ranging from 100 to 0.625 µg/mL. Sterile Erlenmeyer flasks (100mL), each containing 10 mL nutrient broth were sonicated for 10 min after adding the test compound of required concentration. Subsequently, the flasks were inoculated with 1 mL of the freshly prepared bacterial suspension in order to maintain initial bacterial concentration 10^³–10^⁴ CFU mL⁻¹, and then incubated in an orbital shaker at 200 rpm and 37°C. Bacterial growth was measured as increase in absorbance at 600 nm determined using a spectrophotometer. The experiments also included a positive control (flask containing test compound and nutrient media, devoid of inoculum) and a negative control (flask containing inoculum and nutrient media, devoid of test compound). The negative controls indicated the microbial growth profile in the absence of test compounds. The absorbance values for positive controls were subtracted from the experimental values. All the experiments were carried out in triplicate.

**DPPH free radical scavenging activity for antioxidant activity determination**

The working solutions of test compounds and standards were prepared in methanol. Gallic acid solution was used as standard and DPPH solution (0.1mM, 1 mL) as blank. Different concentrations (500-10 µg/mL) of test compounds were pipetted to the test tubes and volume adjusted to 3 mL with methanol. 1 mL of DPPH (0.1mM) solution was mixed with 1 mL of sample and standard solution separately. The samples were vortexed, incubated in dark at RT for 30 min and the absorbance was measured at 517 nm against blank samples in a spectrophotometer. The absorbance was recorded and radical scavenging activity was expressed as percentage inhibition of DPPH radical and was calculated by following equation:

\[
\text{Percent radical scavenging activity} = \left( \frac{\text{Absorbance of control-Absorbance of sample}}{\text{Absorbance of control}} \right) \times 100.
\]
In silico ADMET Prediction

The pharmacokinetic profile of all the active antibacterial and anti-oxidant compounds 10, 11-15, 20, 23-25 along with reference compounds streptomycin and gallic acid was predicted by using programs Qikprop v3.5\textsuperscript{27} and ADMET prediction module of Discovery Studio v2.5\textsuperscript{30}. All the compounds were prepared in neutralized form for the calculation of pharmacokinetic properties by QikProp using Schrodinger’s Maestro Build module and LigPrep, saved in SD format and further used for ADMET analysis using Discovery Studio. The programs QikProp utilizes the method of Jorgensen\textsuperscript{39} whereas ADMET module of Discovery use various ADMET models to compute pharmacokinetic properties and descriptors such as octanol/water partitioning coefficient, aqueous solubility, brain/blood partition coefficient, intestinal wall permeability, plasma protein binding and others.

Conclusions

Several symmetrical and unsymmetrical C5-curcuminoids were synthesized in the present study, some of the compounds (10, 11 and 25) exhibited significant in vitro antibacterial activity against S. aureus and P. aeruginosa. In addition, few analogues (10, 12-15 and 25) also displayed good antioxidant activity with IC\textsubscript{50} values 33.87 to 49.45 µg/mL. ADMET calculations show that compounds 10, 11 and 20-25 have improved oral bioavailability together with high cell permeability values (QPPCaco, QPPPMDCK), which indicates that distribution, metabolism and excretion characteristics of these compounds is better when compared to curcumin. On the basis of these in vitro and in silico results, compounds 20, 23-25 can be taken up as lead molecules for further development. The mechanistic studies depicting mode of action of these compounds, in vitro toxicity studies and structural modification of the lead molecules are under progress and results will be published in due course of time.

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