3. SYNTHESIS AND ANTICANCER ACTIVITY OF GLYCOSIDES OF CURCUMIN AND ISOCURCUMIN

3.1 INTRODUCTION

Curcumin isolated from rhizomes of *Curcuma longa* displays broad range of biological activities.¹,² It is established to be safe, tolerable and non-toxic even upto 8g per day.³ Poor aqueous solubility, rapid metabolism and systemic elimination and hence poor bioavailability have limited the utility of curcumin as a therapeutic agent.⁴ Yang *et al.*, have reported 1% oral bioavailability of curcumin in rat plasma.⁵ This warrants synthesis of more soluble analogues of curcumin.

3.2 LITERATURE REVIEW

There are several strategies adopted to improve solubility of curcumin and hence its bioavailability. These include physical and chemical modifications. Encapsulation of curcumin in oil/water nanoemulsions to enhance the solubility has been reported.⁶ Nanoencapsulation of curcumin using natural biopolymer and non-ionic surfactant to improve its solubility, thus enabling its usage in nutraceuticals has been published.⁷ Solubility of complexes of curcumin with β-cyclodextrin and hydroxypropyl-β-cyclodextrin surpasses that of parent curcumin.⁸ Oral bioavailability of curcumin has been elevated by dripping pill technology.⁹ Water soluble curcumin nanocrystals have been synthesised by solvent exchange method.¹⁰ Curcumin loaded liposomal formulations have been proven to improve bioavailability and also increase stability and anticancer potential on pancreatic adenocarcinoma (PA) cancer cell lines.¹¹ Plasma
concentration of curcumin can be increased and systemic circulation span can be protracted through preparation of micelles.\textsuperscript{12} Solubility of curcumin has been increased by phosphorylation, etherification and esterification and these derivatives of curcumin have been evaluated for their anticancer activity.\textsuperscript{13} Dendrimer-curcumin conjugates are proven to have better solubility and cytotoxicity when tested against breast cancer cell lines.\textsuperscript{14} Curcumin/5-FU loaded thiolated chitosan nanoparticles exhibited enhanced anticancer effect on colon cancer cell line and improved bioavailability \textit{in vivo} as compared to curcumin or 5-FU.\textsuperscript{15} The solubility and stability of curcumin in physiological pH has been improved through preparation of curcumin-hyaluronic acid conjugates.\textsuperscript{16} A review on metal complexes of curcumin discusses about improved bioavailability and biological activity.\textsuperscript{17} Curcumin enaminone analogues are shown to exhibit comparable or better antioxidant and antiproliferative activities and better solubility than curcumin.\textsuperscript{18} Curcumagalactomannoside (CGM) formulations offer seven to ten times more bioavailability of curcumin in humans as compared to unformulated curcumin.\textsuperscript{19}

Enzymatic synthesis of curcumin glucosides has been reported.\textsuperscript{20-23} Chemical synthesis of curcumin mono and diglycosides from glycosylated arylaldehydes has been published.\textsuperscript{24} Synthesis of different bioconjugates of curcumin including curcumin-4,4'-di-O-\textbeta-D-glucopyranoside has been reported and the bioconjugates are proven to act as prodrugs and some of them exhibit enhanced antibacterial and antifungal activities than standard reference drugs. This has been attributed to better cellular uptake or reduced metabolism.\textsuperscript{25} Synthesis of curcumin glucosides using ultrasound assisted biphasic reaction
conditions employing phase transfer catalyst has resulted in improved reaction rates, yield and selectivity.\textsuperscript{26}

Although the above reports represent several methods to synthesise glycosides, these methods have limitations such as lack of process feasibility, expensive reagents, sophisticated reaction conditions, etc. Hence in the present work, efforts were made to modify the existing procedure to synthesise the required glycosides.\textsuperscript{24} Also the synthesis of 2,3-unsaturated glucoside of curcumin and 2-deoxy glucoside of curcumin were taken up.

### 3.3 RESULTS AND DISCUSSION

The following scheme depicts the different steps involved in the synthesis of curcumin -β-di-D-glucoside (7a).

Scheme 3.1  
\begin{enumerate}
\item a) acetobromo glucose / Bu\textsubscript{4}NBr/ CHCl\textsubscript{3}/ 1N NaOH;  
\item b) AADFB / EA / n-BuNH\textsubscript{2} / RT / 24 h;  
\item c) aq. CH\textsubscript{3}OH / reflux / MW / 100-150°C / 3-5 min;  
\item d) CH\textsubscript{3}ONa / dry CH\textsubscript{3}OH / RT / 30 min / Amberlite IR-120 H\textsuperscript{+} resin
\end{enumerate}
2,3,4,6-Tetra-\(O\)-acetyl-\(\alpha\)-D-glucopyranosyl bromide was prepared from \(\alpha\)-D-glucose according to the standard procedure.\(^{27}\) The melting point and NMR spectroscopic data were found to be in accordance with literature report.\(^{26}\)

Vanillin-2,3,4,6-tetraacetyl-\(\beta\)-D-glucoside 2a was prepared by treating vanillin (1a) with 2,3,4,6-Tetra-\(O\)-acetyl-\(\alpha\)-D-glucopyranosyl bromide using reported method.\(^{24}\)

In the present work, difluoroboronite complex of curcumin-\(\beta\)-di-D-glucoside tetraacetate 5a was prepared from vanillin-2,3,4,6-tetraacetyl-\(\beta\)-D-glucoside and AADFB complex. Synthesis of curcumin through formation of difluoroboronite complexes\(^{28}\) has been found to be advantageous over conventional method which uses acetylacetone-B\(2\)O\(3\) complex.\(^{24}\)

Decomplexation of difluoroboronite complex of curcumin-\(\beta\)-di-D-glucoside octaacetate 5a required prolonged refluxing in aqueous methanol.\(^{28}\)

Hence decomplexation was attempted in various solvents like water, DMF, DMSO, acetic acid, 1,2-dichloroethane, ethylene glycol, ethylene glycol monomethyl ether, glycerol, polyethylene glycol under different temperatures and pressures. In none of the above mentioned solvents and conditions, there was appreciable decomplexation (<10%) as evident from work up and separation of products from reaction mixture. Prolonged heating led to decomposition of glucoside. Best result was obtained under microwave conditions using aqueous methanol which resulted in reduced reaction time (3-5 min) and comparable yield.
Deacetylation of curcumin-β-di-D-glucoside tetraacetate was achieved using sodium methoxide in dry methanol to yield curcumin-β-di-D-glucoside 7a.\textsuperscript{26}

Curcumin-β-mono-D-glucoside (9a) was prepared as per the scheme below:

Scheme 3.2 a) acetobromo glucose / Bu\textsubscript{4}NBr/ CHCl\textsubscript{3}/ 1N NaOH; b) FADFB / EA / n-BuNH\textsubscript{2} / RT / 24 h; c) aq. CH\textsubscript{3}OH / reflux / MW / 100-150\degree\textsuperscript{0}C / 3-5 min; d) CH\textsubscript{3}ONa / dry CH\textsubscript{3}OH / RT / 30 min / Amberlite IR-120 H\textsuperscript{+} resin

Curcumin-β-di-D-galactoside and curcumin-β-mono-D-galactoside (8a and 10a respectively) (Table 3.2) were prepared in the same manner as above using acetobromogalactose.

Similarly isocurcumin-β-di-D-glucoside and isocurcumin-β-mono-D-glucoside (7b and 9b respectively) (Table 3.2) and isocurcumin-β-di-D-galactoside and isocurcumin-β-mono-D-galactoside (8b and 10b respectively) (Table 3.2) were synthesised using isovanillin and corresponding sugars.

All the glucosides and galactosides mentioned in the study have been thoroughly characterised by NMR spectroscopy and the yield, melting point and spectral results are in agreement with literature reports.\textsuperscript{24}
The $^1$H NMR spectrum of curcumin-β-D-diglucoside 7a (Table 3.2) is presented (Fig. 3.1) and discussed below:

**Figure 3.1** $^1$H NMR spectrum of curcumin-β-di-D-glucoside (7a) in CDCl$_3$ (500 MHz)
The $^1$H NMR spectrum of curcumin-β-di-D-glucoside 7a exhibited the appearance of signal at $\delta$ 3.84 ppm corresponding to methoxy protons. Two doublets ($J = 12.8$ Hz) at $\delta$ 7.59 ppm and $\delta$ 6.87 ppm correspond to olefinic protons present at β and α position with respect to carbonyl carbon respectively. This shows geometry of double bond to be trans isomer. The enolic proton flanked between two carbonyl groups appears as a singlet at $\delta$ 6.12 ppm. Six protons in the range of $\delta$ 7.12-7.38 ppm correspond to six protons present in the two aromatic rings. Resonance signal of anomeric proton at $\delta$ 5.09 ppm matches with the value reported in the literature for curcumin-β-digluoside.$^{23,26}$ The remaining signals at $\delta$ 3.17-5.29 ppm are due to 22 protons in glucoside moiety. The $^{13}$C NMR spectral data are given in Table 3.1.
Synthesis and Evaluation of Novel Curcumin Conjugates and Hybrids as Potential Anticancer Agents

Figure 3.2 $^{13}$C NMR spectrum of curcumin-β-D-diglucoside (7a) in CDCl$_3$ (125 MHz)
Table 3.1 $^{13}$C NMR spectral data of curcumin-\(\beta\)-D-diglucoside

(7a) in DMSO-\(d_6\) (125 MHz)

<table>
<thead>
<tr>
<th>Carbons</th>
<th>(\delta) (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1 &amp; C-7</td>
<td>140.3</td>
</tr>
<tr>
<td>C-2 &amp; C-6</td>
<td>122.5</td>
</tr>
<tr>
<td>C-3 &amp; C-5</td>
<td>183.2</td>
</tr>
<tr>
<td>C-4</td>
<td>101.2</td>
</tr>
<tr>
<td>Aromatic C</td>
<td>149.1, 148.5, 128.6,</td>
</tr>
<tr>
<td></td>
<td>115.0, 111.3</td>
</tr>
<tr>
<td>Anomeric C</td>
<td>99.6</td>
</tr>
<tr>
<td>Sugar unit C</td>
<td>77.1, 76.8, 73.1, 69.6,</td>
</tr>
<tr>
<td></td>
<td>60.6</td>
</tr>
<tr>
<td>Methoxyl C</td>
<td>55.7</td>
</tr>
</tbody>
</table>
Figure 3.3 DEPT-135 spectrum of curcumin-β-D-diglucoside (7a) in CDCl₃ (125 MHz)
In DEPT (Distortionless Enhancement by Polarisation Transfer) spectrum, the methylene carbon signal was observed at δ 61 ppm.

There are few reports on biological activities of curcumin glycosides reported in the literature which include anticancer, \(^{30-33}\) antibacterial, \(^{34}\) antiallergic \(^{35}\) and antihypertensive. \(^{36}\)

Figure 3.4 Structures of curcumin glycosides (7a, 9a, 8a & 10a), isocurcumin glycosides (7b, 9b, 8b & 10b), curcumin (11), isocurcumin (12), tetramethoxy curcumin (13) and curcumin diacetate (14)

*In vitro* cytotoxicity studies of glycosides of curcumin and isocurcumin were determined by MTT assay and the results are tabulated:
Table 3.2 Cytotoxicity studies (MTT assay) of di and mono glycosyl curcums (7a, 9a, 8a & 10a) and isocurcums (7b, 9b, 8b & 10b), curcumin (11), isocurcumin (12), tetramethoxy curcumin (13) and diacetyl curcumin (14)

<table>
<thead>
<tr>
<th>Compound Number</th>
<th>R_1</th>
<th>R_2</th>
<th>R_3</th>
<th>R_4</th>
<th>IC_{50} values (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MIA PaCa-2</td>
</tr>
<tr>
<td>7a</td>
<td>-OCH_3</td>
<td>-O-Glu</td>
<td>-OCH_3</td>
<td>-O-Glu</td>
<td>&gt;100</td>
</tr>
<tr>
<td>9a</td>
<td>-OCH_3</td>
<td>-OH</td>
<td>-OCH_3</td>
<td>-O-Glu</td>
<td>&gt;100</td>
</tr>
<tr>
<td>8a</td>
<td>-OCH_3</td>
<td>-O-Gal</td>
<td>-OCH_3</td>
<td>-O-Gal</td>
<td>&gt;100</td>
</tr>
<tr>
<td>10a</td>
<td>-OCH_3</td>
<td>-OH</td>
<td>-OCH_3</td>
<td>-O-Gal</td>
<td>&gt;100</td>
</tr>
<tr>
<td>7b</td>
<td>-O-Glu</td>
<td>-OCH_3</td>
<td>-O-Glu</td>
<td>-OCH_3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>9b</td>
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<td>-OCH_3</td>
<td>-O-Glu</td>
<td>-OCH_3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>8b</td>
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<td>-OCH_3</td>
<td>-O-Gal</td>
<td>-OCH_3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>10b</td>
<td>-OH</td>
<td>-OCH_3</td>
<td>-O-Gal</td>
<td>-OCH_3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>11</td>
<td>-OCH_3</td>
<td>-OH</td>
<td>-OCH_3</td>
<td>-OH</td>
<td>&gt;100</td>
</tr>
<tr>
<td>12</td>
<td>-OH</td>
<td>-OCH_3</td>
<td>-OH</td>
<td>-OCH_3</td>
<td>47.74±3.89</td>
</tr>
<tr>
<td>13</td>
<td>-OCH_3</td>
<td>-OCH_3</td>
<td>-OCH_3</td>
<td>-OCH_3</td>
<td>&gt;100</td>
</tr>
<tr>
<td>14</td>
<td>-OCH_3</td>
<td>-OAc</td>
<td>-OCH_3</td>
<td>-OAc</td>
<td>37.4±2.81</td>
</tr>
</tbody>
</table>

Mono and di glycosides of curcumin and isocurcumin synthesised in the present study were screened on a panel of cancer cell lines (MIA PaCa-2, SKOV-3, BMG-1). Aglycones (curcumin and isocurcumin) were also screened on the same panel of cell lines. From the Table 3.2, it can be seen that curcumin (11) exhibits an IC_{50} value of 53.45±2.64 µM and 44.9±3.3 µM on SKOV-3 and BMG-1 cell lines respectively but was not active up to 100 µM on MIA PaCa-2 cell line. But isocurcumin (12) which differs from curcumin in the position of phenolic hydroxyl and methoxy groups attached to aromatic ring was active on all the three cell lines. Isocurcumin was more potent than curcumin with an IC_{50} value of 47.74±3.89 µM on MIA PaCa-2 but less potent than curcumin with an IC_{50} value of 83.56±3.3 µM on SKOV-3 and 78.94±2.94 µM on BMG-1. Although all the glycosides displayed better aqueous solubility compared to aglycones, they were not active up to 100 µM (Table 3.2).
The requirement of presence of phenolic hydroxyl group for cytotoxicity was analysed by preparing other conjugates of curcumin like tetramethoxy curcumin and diacetyl curcumin. These conjugates were screened on the same panel of cell lines. Tetramethoxy curcumin was not active upto 100 μM on all the three cell lines whereas diacetyl curcumin was active. In fact, the diacetyl derivative was more potent than curcumin and isocurcumin on MIA PaCa-2 with an IC₅₀ value of 37.4±2.81 μM. From the observation, it is clear that upon glycosylation, there is a decrease in activity on the tested cell lines. Also the position of phenolic hydroxyl and methoxy group impacts the activity which is evident from comparison of IC₅₀ values of curcumin and isocurcumin though there is no regular trend. However the synthesis of tetramethoxy and diacetyl conjugates led to incorrigible results and hence warrants synthesis and screening of series of more conjugates.

Ferrier rearrangement reaction involves nucleophilic substitution combined with allylic shift. It results in the formation of 2,3-unsaturated glycosides.

Scheme 3.3 Ferrier rearrangement of alcohols/phenols
Ferrier rearrangement was attempted with vanillin as the starting material using various Lewis acid catalysts like BF$_3$, AlCl$_3$, ZnCl$_2$, Montmorillite K-10, Indium chloride, LiBF$_4$, LiClO$_4$, SnCl$_4$, etc in different solvents and at different temperatures. The reaction did not yield the desired 2,3-unsaturated glycosides at room temperature. Upon refluxing for few hours, there was no progress in the reaction, instead it led to decomposition of tri-O-acetyl-D-glucal. Attempt to perform Ferrier rearrangement directly on curcumin also led to the same observation. Though Ferrier rearrangement of curcumin using ferric sulphate catalyst has been reported in a research study (K. S. Parvathy , Chemical approach toward preparation of water soluble curcumin derivatives, Department of Plantation Products, Spices and Flavour Technology, CFTRI, Mysore, India, Chapter 1, Page No. 150), the reaction could not be reproduced in our lab with curcumin or vanillin. Hence more experiments with new catalysts are needed to synthesise 2,3-unsaturated glycosides.
3.4 CONCLUSION

Curcumin and isocurcumin glycosides were synthesised from vanillin and isovanillin respectively using an improved procedure and all the products were purified and thoroughly characterised using NMR spectroscopic techniques. All the compounds had excellent aqueous solubility. All the glycosides were screened on a panel of representative cell lines (MIA PaCa-2, SKOV-3 and BMG-1). But the glycosides did not exhibit cytotoxic effect up to 100 µM. Hence in order to study the essentiality of presence of phenolic –OH on cytotoxicity, few derivatives like tetramethoxy and diacetyl derivatives of curcumin were synthesised and screened on the same panel of cell lines but cytotoxicity studies did not lead to corrigeble results and further synthesis of new conjugates and biological studies are required to draw solid conclusions though the glycosides were prepared using improved conditions and possessed better solubility.

3.5 EXPERIMENTAL

3.5.1 Procedure for preparation of α-acetobromo-D-glucose

Acetic anhydride (14.5 g, 142 mmol) was stirred at 4°C in a three neck RB flask fitted with an overhead magnetic stirrer. To this, perchloric acid (0.1 ml) was added slowly through a micro syringe maintaining the temperature below 4°C during addition. The mixture was stirred for a few minutes at room temperature. To this, α-D-glucose (3.5 g, 19.4 mmol) was then added portionwise with constant stirring maintaining the temperature between 30-40°C during addition. After cooling the reaction mixture to about 20°C, red phosphorus (1.25g, 40 mmol) was added portionwise. This was followed by dropwise addition of bromine maintaining the temperature below 20°C. Water (1.2 ml) was added dropwise over
a period of 15-20 minutes maintaining the temperature below 20°C. The reaction mixture was stirred at room temperature for about 2 hours and then diluted with dichloromethane (50 ml) and filtered through glass wool. The organic layer is then stirred in a beaker with portionwise addition of solid sodium bicarbonate till the effervescence ceased. The contents were then transferred into a separating funnel (1 L) and organic layer was separated and washed once with ice-cold saturated solution of sodium bicarbonate and then with ice-cold distilled water. The organic layer is then dried over anhydrous sodium sulphate and the solvent removed under vacuum carefully maintaining the temperature below 40°C. The gummy mass obtained was crystallised using diethyl ether : hexane mixture to give a white solid which was filtered under nitrogen atmosphere and stored under nitrogen atmosphere in an air tight amber coloured bottle below 4°C in a refrigerator.

3.5.2 Preparation of Tetra-O-acetyl-β-D-glucopyranosyl vanillin (2a)²⁴

To stirred solution of vanillin (1.52 g, 10 mmol) in chloroform (20 ml), 1 N NaOH (20 ml), 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl bromide (4.112 g, 10 mmol) and tetrabutyl ammonium bromide (3.27 g, 10 m mol) were added at room temperature. The resulting mixture was stirred vigorously for an hour using an overhead magnetic stirrer. After diluting the reaction mixture with ethyl acetate (20-30 ml), the organic layer was separated and washed with 1N NaOH, ice-cold water and ice-cold saturated brine solution. The organic layer was then dried over anhydrous sodium sulphate and solvent removed under vacuum. The crude solid was recrystallised from ethanol to give colourless solid.
3.5.3 Preparation of difluoroboronite complex of curcumin-β-di-D-glucoside octaacetate (3a)

Tetra-O-acetyl-β-D-glucopyranosyl vanillin (1.52 g, 10 mmol) was stirred in ethyl acetate (10 ml). To this was added acetylacetonedifluoroboronite complex (0.739 g, 5 mmol) in small portions with constant stirring at room temperature. n-butyl amine (0.065 g, 0.9 mmol) in 5 ml ethyl acetate was added very slowly over a period of 30 minutes. The reaction was then stirred at room temperature for about 3-4 hours and left overnight at room temperature. The solvent was removed under vacuum. To the solid obtained, water was added and then filtered. The crude solid was purified by crystallisation from hexane : ethyl acetate.

Same procedure was used to prepare difluoroboronite complexes of curcumin-β-di-D-galactoside octaacetate (4a), isocurcumin-β-di-D-glucoside octaacetate (3b) isocurcumin-β-di-D-galactoside (4b).

3.5.4 Preparation of difluoroboronite complex of curcumin-β-mono-D-glucoside tetraacetate (5a)

Tetra-O-acetyl-β-D-glucopyranosyl vanillin (1.52 g, 10 mmol) was stirred in ethyl acetate (10 ml). To this was added feruloylacetylacetonedifluoroboronite complex (2.82 g, 10 mmol) in small portions with constant stirring at room temperature. n-butyl amine (0.065 g, 0.9 mmol) in 5 ml ethyl acetate was added very slowly over 30 minutes. The reaction was then stirred at room temperature for about 3-4 hours and left overnight at room temperature. The solvent was
removed under vacuum. To the solid obtained, water was added and then filtered. The crude solid was purified by crystallisation from hexane : ethyl acetate.

Same procedure was used to prepare difluoroboronite complexes of curcumin-β-mono-D-galactoside tetraacetate (6a), isocurcumin-β-mono-D-glucoside tetraoctaacetate (5b) isocurcumin-β-mono-D-galactoside (6b).

**3.5.5 Procedure for decomplexation of difluoroboronite complex of curcumin-β-diglucoside octaacetate (3a)**

The difluoroboronite complex of curcumin-β-di-D-glucoside octaacetate (3a) was dissolved in aqueous methanol (MeOH : water - 9:1) and irradiated under microwave conditions at 150°C for about 3 minutes. Solvent was removed under vacuum and the resulting solid was washed thoroughly with water and dried. A small portion of the crude solid was purified by column chromatography whereas the remaining was subjected to deacetylation without further purification. Same procedure was followed for all decomplexation reactions.

**3.5.6 Procedure for preparation of curcumin- β-di-D-glucoside (7a)**

Curcumin-β-di-D-glucoside octaacetate (0.1 g) was dissolved in dry methanol (1 ml). To this, sodium methoxide (prepared by dissolving 4.5 mg of sodium in 0.6 ml dry methanol) was added dropwise and the mixture was stirred for about 30 minutes. Completion of reaction was monitored by TLC. The reaction mixture was neutralised by freshly prepared Dowex (IR-120) H⁺ resin and the resin was filtered and the solvent removed under vacuum to afford pure curcumin β-diglucoside as orange solid. Same procedure was used to prepare all monoglycosides and diglycosides reported in the study.
3.5.7 Spectral data of glycosides

CURCUMIN-β-DI-D-GLUCOSIDE (7a): Orange powder; m.p. 155-157 °C; \(^1\)H NMR (DMSO- \(d_6\), 500 MHz), \(\delta\) (ppm): 7.59 (d, 2H, \(J = 16\) Hz), 7.38 (s, 2H), 7.25 (d, 2H, \(J = 8\) Hz), 7.13 (d, 2H, \(J = 8.5\) Hz), 6.87 (d, 2H, \(J = 16\) Hz), 6.12 (s, 1H), 5.29 (bs, 2H), 5.09 (s, 2H), 5.02 (t, 4H), 4.55 (bs, 2H), 3.84 (s, 6H), 3.68 (m, 3H), 3.17-3.45 (m, 8H); \(^{13}\)C NMR (DMSO- \(d_6\), 100 MHz), \(\delta\) (ppm): 183.2, 149.1, 148.5, 140.3, 128.6, 122.5, 115, 111.3, 101.2, 99.6, 77.1, 76.8, 73.1, 69.6, 60.6, 55.7.

CURCUMIN-β-DI-D-GALACTOSIDE (8a): Orange powder; m.p. 155-159 °C; \(^1\)H NMR (DMSO- \(d_6\), 400 MHz), \(\delta\) (ppm): 7.59 (d, 2H, \(J = 15.9\) Hz), 7.38 (s, 2H), 7.25 (d, 2H, \(J = 8.4\) Hz), 7.13 (d, 2H, \(J = 8.4\) Hz), 6.85 (d, 2H, \(J = 15.9\) Hz), 6.13 (s, 1H), 5.07 (bs, 2H), 4.96 (d, 2H, \(J = 7.5\) Hz), 4.5 (bs, 4H), 3.84 (s, 6H), 3.2-3.7 (m, 14H); \(^{13}\)C NMR (DMSO- \(d_6\), 100 MHz), \(\delta\) (ppm): 183.2, 149.2, 148.6, 140.3, 128.5, 122.4, 115.1, 111.4, 101.1, 100.2, 75.5, 73.5, 70.1, 68.1, 60.3, 55.8.

CURCUMIN-β-MONO-D-GLUCOSIDE (9a): Orange powder; m.p. 110-113 °C; \(^1\)H NMR (DMSO- \(d_6\), 400 MHz), \(\delta\) (ppm): 7.59 (d, 2H, \(J = 15.9\) Hz), 7.38 (s, 2H), 7.25 (d, 2H, \(J = 8.4\) Hz), 7.13 (d, 2H, \(J = 8.4\) Hz), 6.85 (d, 2H, \(J = 15.9\) Hz), 6.13 (s, 1H), 5.2 (bs, 1H), 5.06 (bs, 1H), 4.98 (d, 1H, \(J = 5.6\) Hz), 4.94 (d, 1H, \(J = 7.6\) Hz), 4.5 (m, 1H), 4.3 (bs, 1H), 3.84 (s, 6H), 3.1-3.6 (m, 5H); \(^{13}\)C NMR (DMSO- \(d_6\), 100 MHz), \(\delta\) (ppm): 184.6, 182.8, 148.9, 148.6, 148, 147.4, 141.6, 140.2, 129.5, 126.4, 123.9, 122.6, 121.4, 116.2, 115.8, 111.5, 111.2, 101.8, 100.4, 76.1, 74.6, 70.4, 67.6, 60.9, 56.8.
Synthesis and Anticancer Activity of Glycosides of Curcumin and Isocurcumin

CURCUMIN-β-MONO-D-GALACTOSIDE (10a): Orange powder; m.p. 110-112 °C; 'H NMR (DMSO- d6, 400 MHz), δ (ppm): 7.58 (d, 2H, J = 15.6 Hz), 7.1-7.4 (m, 6H), 6.79 (d, 2H, J = 15.6 Hz), 6.1 (s, 1H), 5.16 (bs, 1H), 4.5-5 (m, 4H), 3.8 (s, 6H), 3.5-3.6 (m, 6H); 13C NMR (DMSO- d6, 100 MHz), δ (ppm): 184.3, 183, 149.9, 149.6, 149, 148.4, 141.5, 140.5, 129, 126.7, 123.7, 122.9, 121.5, 116.1, 115.4, 111.7, 111.6, 101.5, 100.6, 76, 74, 70.6, 68.6, 60.8, 56.1.

ISOCURCUMIN-β-DI-D-GLUCOSIDE (7b): Orange powder; m.p. 156-158 °C; 'H NMR (DMSO- d6, 400 MHz), δ (ppm): 7.56 (d, 2H, J = 15.6 Hz), 7.53 (s, 2H), 7.32 (d, 2H, J = 8 Hz), 7.05 (d, 2H, J = 8.4 Hz), 6.79 (d, 2H, J = 15.6 Hz), 6.03 (s, 1H), 5.15 (d, 3H, J = 5.6 Hz), 5.00 (d, 3H, J = 7.2 Hz), 4.91 (d, 3H, J = 5.6 Hz), 4.77 (t, 3H), 4.56 (d, 3H, J = 4.4 Hz), 4.07 (bs, 1H), 3.8 (s, 6H), 3.5-3.7 (m, 6H); 13C NMR (DMSO- d6, 100 MHz), δ (ppm): 183.6, 151.6, 147.2, 140.9, 127.9, 124.2, 122.5, 114.6, 112.7, 101.9, 100.9, 76.1, 74.1, 70.6, 68.8, 61.0, 56.1.

ISOCURCUMIN-β-DI-D-GALACTOSIDE (8b): Orange powder; m.p. 158-160 °C; 'H NMR (DMSO- d6, 400 MHz), δ (ppm): 7.48 (d, 2H, J = 15.6 Hz), 7.44 (s, 2H), 7.23 (d, 2H, J = 8 Hz), 6.96 (d, 2H, J = 8.4 Hz), 6.72 (d, 2H, J = 15.6 Hz), 5.94 (s, 1H), 5.22 (d, 2H, J = 4.8 Hz), 4.95-5.06 (m, 6H), 4.59 (bs, 2H), 3.74 (s, 6H), 3.1-3.4 & 3.62-3.74 (m, 12H); 13C NMR (DMSO- d6, 100 MHz), δ (ppm): 183.6, 151.5, 147.2, 140.8, 127.9, 124.4, 122.5, 114.4, 112.7, 102.1, 100.3, 77.6, 77.5, 73.6, 70.3, 61.2, 56.2.

ISOCURCUMIN-β-MONO-D-GLUCOSIDE (9b): Orange Powder; mp: 112-114 °C; 'H NMR (DMSO- d6, 400 MHz), δ (ppm): 7.59 (d, 2H, J = 15.6 Hz), 7.54 (d, 1H, J = 1.6 Hz), 7.31 (dd, 1H, J = 8 Hz, J' = 2 Hz), 7.34 (d, 1H, J = 1.6
Synthesis and Anticancer Activity of Glycosides of Curcumin and Isocurcumin

ISOCURCUMIN-β-MONO-D-GALACTOSIDE (10b): Orange Powder; mp: 110-112 °C; ¹H NMR (DMSO- d₆, 400 MHz), δ (ppm): 7.63 (d, 1H, J = 15.6 Hz), 7.62 (d, 1H, J = 15.6 Hz), 7.17-7.43 (m, 6H), 6.87 (d, 2H, J = 15.6 Hz), 6.15 (s, 1H), 5.23 (d, 1H, J = 4.4 Hz), 5.02 (d, 1H, J = 7.6 Hz), 4.96 (bs, 1H), 4.74 (bs, 1H), 4.61 (d, 1H, J = 3.6 Hz), 3.89 (s, 3H), 3.88 (s, 3H), 3.1-3.4 (m, 6H); ¹³C NMR (DMSO- d₆, 100 MHz), δ (ppm): 183.8, 182.3, 149.4, 148.6, 148.3, 141.5, 140.3, 129.1, 126.9, 123.6, 122.9, 121.5, 116, 115.4, 111.4, 101.5, 100.4, 75.7, 73.3, 70.4, 68.4, 60.7, 56.1

3.5.8 Materials - Biology

Growth medium Dulbecco’s Modified Eagle’s Medium (DMEM), Fetal Bovine Serum (FBS), were purchased from Himedia Laboratories. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was purchased from Sigma. Dimethyl sulphoxide (DMSO) (spectroscopic grade) was purchased from Spectrochem.

3.5.9 Cell viability assay (MTT)

The cells were cultured in DMEM media containing 10% fetal bovine serum (FBS) at 37°C and 5% CO₂. In total, 20,000 cells were seeded in each well
(in 96-well plates) containing 200µl of DMEM medium. After 24h, different test concentrations of the glycosides were added, and again after 24 h of treatment, 20µl per well of MTT (5 mg/ml; stock solution, Sigma) was added. The plates were incubated at 37°C for additional 4 h. The medium was discarded and the formazan, which formed in the cells, was dissolved with 200 µl of DMSO. The intensity of colour production was measured at 570nm and 695nm in a spectrophotometer. IC50 values were calculated using Microsoft Excel for semilog curve fitting with regression analysis.
3.6 REFERENCES


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