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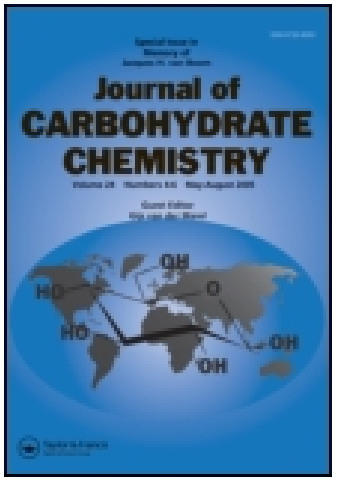

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Synthesis of a Novel Series of (E,E)-4,6-bis(styryl)-2-O-Glucopyranosyl-Pyrimidines and Their Potent Multidrug Resistance (MDR) Reversal Activity Against Cancer Cells

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Synthesis of a Novel Series of (*E*,*E*)-4,6-bis(styryl)-2-*O*-Glucopyranosyl-Pyrimidines and Their Potent Multidrug Resistance (MDR) Reversal Activity Against Cancer Cells

Lei Gao,[1] Qian Liu,[1] Sumei Ren,[1] Shengbiao Wan,[1] Tao Jiang,[1] Iris L. K. Wong,[2] Larry M. C. Chow,[2] and Shixi Wang[3]

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A novel series of methoxy or benzyloxy substituted (*E*,*E*)-4,6-bis(styryl)-2-*O*-glucopyranosyl-pyrimidines as curcuminoid analogs were synthesized in four steps with total yields of 21.5% to 33.9%. A549 and HL60 cells were employed for the anticancer activity testing. The results demonstrated that **5a**, **5c**, and **5e** have some inhibitory activity against the HL-60 cell line. Unfortunately, no compound displayed inhibitory activity against A549 except for **5c**. MDR reversal activity results demonstrated that compounds **4a** (RF = 12.3) and **4b** (RF = 18.5) showed strong reversal activity to the P-gp-mediated LCC6MDR cells compared to verapamil (RF = 3.2) and no cytotoxicity to cancer or normal cell lines even at a high concentrations (100 μM).

Keywords Curcumin; Pyrimidine; Glucosylation; Anticancer; MDR modulator

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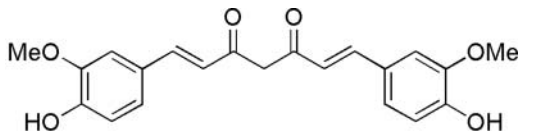
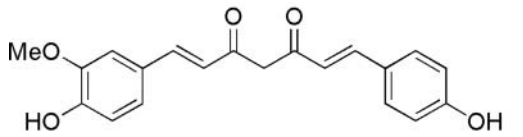
INTRODUCTION

Curcuminoids as dietary polyphenolic compounds are of interest since tumeric plants are often used as herbal medicines.[1] Curcumin (curcumin I), demethoxycurcumin (curcumin II), and bis-demethoxycurcumin (curcumin III) are the major forms of curcuminoids (Fig. 1) found in turmeric powder, which exhibit anticancer, antioxidant, and anti-inflammatory activities.[2] Evidence has also been found that curcumin has hepatoprotective and nephroprotective activities, can suppress thrombosis and protect against myocardial infarction, and has hypoglycemic and antirheumatic properties. Most importantly, curcumin has been shown in various animal models and human studies to be extremely safe even at very high doses.[3] Owing to their pharmacological effects and excellent safety profile, curcuminoids have been investigated as lead compounds for treatment of human diseases.[1]

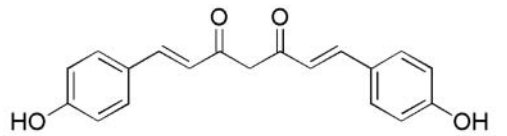
However, the utility of curcumin as a therapeutic agent is limited by its low water solubility and bioavailability, poor stability, and fast metabolism that results in rapid systematic elimination. It is therefore necessary to administer a high dose to achieve a significant intracellular concentration.[4] The phenolic group and the 1,3-dicarbonyl moiety can be degraded via oxidative and hydrolytic pathways both *in vitro* and *in vivo*.[5,6] Many strategies have been used to improve the bioavailability of curcumin, such as protection of the phenolic groups and substitution of the 1,3-dicarbonyl moiety, which have been shown to enhance the curcuminoids' stability and result in increased bioavailability.[6–8]



curcumin I



curcumin II



curcumin III

Figure 1: Structures of major curcuminoids.

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The nitrogen element plays an important role in many drugs, as it is easy to form water-soluble salts with acids and improve the water solubility of the drugs. Therefore, it is of great significance to introduce nitrogen into the structure when designing the structure modification of natural products. It has been found that an increased cytotoxicity can be provided by incorporating an α,β-unsaturated keto group into a nitrogen heterocyclic ring.[9]

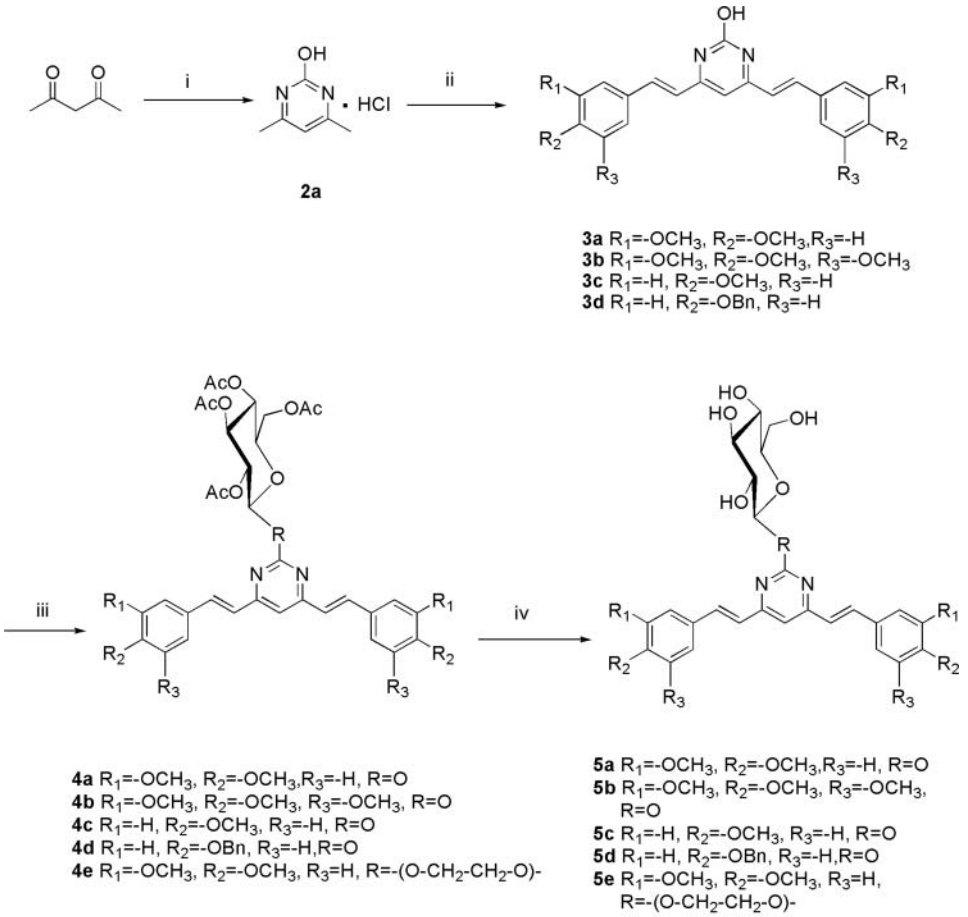
Glucose as a ubiquitous fuel in biology is used as an energy source in most organisms, from bacteria to humans. Glycosylation allows water-insoluble and unstable organic compounds to be converted into the corresponding water-soluble and stable compounds, which could probably improve their bioavailability and pharmacological properties. Furthermore, glycosides of physiologically active compound (i.e., vitamin glycosides) have been reported to be useful antiallegic agents.[10] Many sugar-modified curcumin derivatives have been synthesized by different methods, including enzymatic glycosylation[11] and phase-transfer catalyst glycosylation.[6] While these glycosylation reactions mainly occurred on the hydroxyl of the curcumin aromatic ring, there is no report on the glycosylation of the pyrimidine ring. We herein report the preparation of a series of novel curcumin analogs by incorporating the 1,3-dicarbonyl into 2-hydroxyl pyrimidine, followed by glycosylation of the hydroxyl. The resulting methoxy- or benzyloxy-substituted (*E*,*E*)-4,6-bis(styryl)-2-*O*-glucopyranosyl-pyrimidines and (*E*,*E*)-4,6-bis-(3,4- dimethoxystyryl)-2-(2-*O*-glucopyranosyl)ethyloxgyl-pyrimidines were evaluated *in vitro* using HL-60 and A549 cell lines.

Chemotherapy plays an important role in the treatment of cancer, but the emergence of multidrug resistance (MDR) has made many of the currently available chemotherapeutic agents ineffective.[12] To continue our preliminary research on development of new MDR modulators from natural products,[13] the synthetic compounds were assessed for MDR-reversing activity mediated by P-gp, BCRP, and MRP1 in three different multidrug resistance cancer cell lines. Here we report the synthesis of this novel series of curcumin analogs and their potent multidrug resistance reversal activity against cancer cell lines.

RESULTS AND DISCUSSION

Syntheses of Target Compounds 5a–e

The synthesis of the target compounds **5a**–**e** is outlined in Scheme 1. First, 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (**1a**) as a glycosyl donor was synthesized in one-pot as previously reported.[14] 1-*O*-Bromoethy-2,3,4,6,-tetra-O-acetyl-β-D-glucopyranose (**1b**) was synthesized as described in the Experimental section. 4,6-Dimethyl-2-hydroxyl-pyrimidine hydrochloride (**2a**)



Scheme 1: Synthetic route of compounds 4a-4e, 5a-5e.

was prepared by a reported procedure, in which 2,4-pentanedione and urea were refluxed in anhydrous ethyl alcohol and catalyzed by HCl.[15] Mixtures of **2a** and differently substituted benzaldehydes were then refluxed in anhydrous ethyl alcohol and toluene in the presence of concentrated HCl as catalyst to give **3a–d** with yields of 65% to 70%. To improve the yield, the water generated during the reaction was removed by using a Dean-Stark trap. It is worth noting that 4-(benzyloxy) benzaldehyde was prepared from 4-hydroxybenzaldehyde and benzyl bromide following the reported method.[16] Subsequently, intermediates **4a–e** were synthesized by using tetrabutyl ammonium bromide as the phase-transfer catalyst, which can facilitate the migration of **3a–d** from the

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aqueous phase into the organic phase containing **1a** or **1b** in chloroform. The use of a phase-transfer catalyst in heterogeneous reactions can result in faster reactions and higher conversions or yields. Thus, the yields of this reaction increased from about 20% to about 50% as compared to the common method. For the last step, compounds **4a–e** were deacetylated by a catalytic amount of CH_3ONa in methanol to obtain target compounds **5a–e** with yields of 80% to 85% after recrystallization in methanol. All the compounds were characterized by ^1H NMR, ^{13}C NMR, and HRMS spectrometry.

Cytotoxicity Evaluation

The anticancer activity of compounds **5a–e** against A549 and HL-60 cell lines *in vitro* was determined by the MTT assay and compared with those of the parent compounds curcumin I and curcumin III. Preliminary *in vitro* results demonstrated that most of the synthesized curcumin analogs showed low or moderate inhibition of A549 and HL-60 cells at the concentration of 10 μg/mL as shown in Table 1. Among the analogs, **5a**, **5c**, and **5e** showed slightly higher inhibitory activity against the HL-60 cell line (11.02%, 12.51%, and 16.75%, respectively) when compared with curcumin I and III (8.39% and −6.84%, respectively). However, no inhibition was observed with any of the compounds except for **5c** (8.31%) toward A549.

Evaluating the MDR Reversal Activity of Compound 4a–e

We employed three drug resistance cell lines in this study: (1) a P-glycoprotein (P-gp) overexpressing human breast cancer cell line, LCC6MDR, which displayed 70.5-fold resistance to paclitaxel (IC50 = 183.2 ± 6.3 nM) compared with parental LCC6 cells (IC50 = 2.6 ± 0.5 nM);[13] (2) a BCRP-transfected human embryonic kidney cell line, HEK293/R2, which was 25.2-fold more resistant to topotecan (IC50 = 563.4 ± 41.1 nM) than the parental HEK293/pcDNA3.1 cell line (IC50 = 22.4 ± 2.4 nM);[17] and (3) an MRP1-transfected ovarian cancer cell line, 2008/MRP1, which was 9.2-fold more resistant to DOX (IC50 = 577.1 ± 39.4 nM) than parental 2008/P cells (IC50 = 63.5

Table 1: Inhibition effect of target compounds **5a–e** at 10 μg/mL toward A549 and HL-60 cell lines *in vitro*

Compounds	Inhibition of A549 (%)	Inhibition of HL-60 (%)
Curcumin I	−24.91	8.39
Curcumin III	−21.94	−6.84
5a	−30.83	11.02
5b	−14.00	4.29
5c	8.31	12.51
5d	−29.18	−1.67
5e	−14.68	16.75

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Table 2: MDR reversal activity of **4a–e**

	LCC6MDR		HEK293/R2		+2008MRP1	
Cpds 1 μM	Paclitaxel IC_{50} (nM)	RF	Topotecan IC_{50} (nM)	RF	DOX IC_{50} (nM)	RF
Control	140.0	1.0	612.5	1.0	395.8	1.0
Verapamil	43.8	3.2			73.3	5.4
Ko143			30.5	20.3		
Curcumin I	107.7	1.3	417.2	1.3	439.8	0.9
Curcumin III	107.2	1.3	610.5	1.0	494.8	0.8
4a	11.4	12.3	82.4	7.4	234.6	1.7
4b	7.6	18.5	56.0	10.9	201.2	2.0
4c	106.2	1.3	135.1	4.5	362.0	1.1
4d	195.6	0.7	403.4	1.5	441.2	0.9
4e	91.8	1.5	117.6	5.2	365.0	1.1

[a] IC_{50} values were determined for paclitaxel, topotecan, and DOX with 1.0 μM of curcumin analogs using LCC6MDR, HEK293/R2, and 2008/MRP1 cells. The Relative Fold (RF) represents the fold change of drug sensitivity in different cell lines. RF-(IC_{50} without modulator)/(IC_{50} with modulator) N- 3 independent experiments were performed, and averaged IC_{50} values are presented. No modulator was used in LCC6MDR, HEK293/R2, and 2008/MRP1 cells and the IC_{50} value from this control is used for normalization (RF = 1.0). Verapamil was used at 20 μM for testing MRP1-modulating activity.

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$\pm$ 5.1 nM).[18] Verapamil is a well-known P-gp and MRP1 inhibitor, and Ko143 is a BCRP-specific modulator. Here, we employed these two positive controls in the cell proliferation assay. The MDR reversal activity of synthetic curcumin analogs is listed in Table 2.

Since the preliminary research results showed that introduction of a nonpolar and hydrophobic group into the molecule enhanced the potency of MDR reversal activity,[13,17] a relative low and safe concentration of compounds **4a–e** (1 μM) was used in the assay. All of the compounds displayed no cytotoxicity toward LCC6, LCC6MDR, and normal mouse connective tissue fibroblast (L929) even at the concentration of 100 μM. (Table 3).

The reversal MDR activities of curcumin analogs were compared by measuring the relative fold (RF), defined as a ratio of IC50 without modulator to IC50 with modulator. Table 2 demonstrates the reversal activities of P-gp-, BCRP-, and MRP1-mediated MDR in three different cell lines. Worth noting, to the P-gp-mediated LCC6MDR cell lines, compound **4b** displayed the highest modulating activity with an RF of 18.5 (Table 2). Besides compound **4b**, **4a** also demonstrated strong modulating activity with an RF of 12.3. Verapamil as the well-known P-gp modulator displayed a modulate activity with an RF of 3.2 (Table 2), and curcumin I and curcumin III gave relative low modulating activity with an RF of 1.3 (Table 2). To the BCRP-mediated HEK293/R2 cell lines, the same results were obtained. Compound **4b** (RF = 10.9) and **4a** (RF = 7.4) demonstrated promising modulating activity, though it is lower than

Table 3: *In vitro* cytotoxicity of **4a–e** to LCC6, LCC6MDR, and L929 cell lines[a]

Compounds	IC_{50} (μM)		
	LCC6	LCC6MDR	L929
Curcumin I	19.8 ± 3.4	18.0 ± 2.2	25.0 ± 2.0
Curcumin III	>100	>100	>100
4a	>100	>100	>100
4b	>100	>100	>100
4c	>100	>100	>100
4d	>100	>100	>100
4e	>100	>100	>100

[a]The IC_{50} values were determined after exposure to a series of concentrations of synthetic curcumin analogs using LCC6, LCC6MDR, and L929. L929 is a mouse connective tissue fibroblast cell line. Each experiment has been repeated one to three times with the data presented as the mean ± standard error of mean.

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Ko143. All the results indicate that the presence of a methoxy substituent is needed for inhibiting the functionality of P-gp and BCRP. Moreover, the number of methoxy substituents on the phenyl group is also an important factor for determining the P-gp- and BCRP-modulating activity. Meanwhile, the length of the linker also plays an important role in modulating P-gp and BCRP activity. The compound **4a** with a shorter linker at the 2-position of pyrimidine displayed higher modulating activity than **4e**. Unfortunately, compounds **4a–e** displayed no activity in modulating MRP1-mediated MDR cell lines except for **4b** with an RF of 2.0. At a high concentration (20 μM), verapamil completely reversed DOX resistance in 2008/MRP1 (RF = 5.4 in Table 2). This indicates that the MRP1 modulator maybe has its own special pharmacophore. It is well known that P-gp is thought to transport neutral or positively charged compounds, whereas MRP1 is a transporter of neutral and anionic compounds such as glutathione-conjugated drugs, sulphates, and glucuronidates.[19] This wide difference in substrate specificity of various ABC transporters may explain why our curcumin pyrimidine derivatives markedly exhibit different potency against P-gp, BCRP, and MRP1.

EXPERIMENTAL

General

All reagents used were commercially available. Solvents were treated using standard techniques. Thin-layer chromatography (TLC) was performed on precoated E. Merck silica-gel 60 F254 plates. Column chromatography was

performed on silica gel (200–300 mesh Qingdao China). Melting points were determined on a Mitamura-Riken micro-hot stage and were not corrected. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Jeol JNM-ECP 600 spectrometer with tetramethylsilane (Me_4Si) as the internal standard, and chemical shifts were recorded in δ values. Mass spectra were recorded on a Q-TOF Global mass spectrometer.

Synthesis of 1-O-Bromoethy-2,3,4,6,-tetra-O-acetyl–β-D-Glucose (1b)

Compound 1 (6.0 g, 15.5 mmol) and 2-bromoethanol (1.35 mL 19 mmol) were dissolved in dry CH_2Cl_2 (27 mL), stirring at 0°C under N_2 atmosphere. Then boron trifluoride ethyl ether (10 mL) was added dropwise to the solution at 0°C in the darkness over a period of 20 min. The reaction mixture was allowed to stir for 1.5 h at 0°C and for a further 20 h at rt. After completion of the reaction, the mixture was poured into ice water (45 mL) and extracted with CH_2Cl_2 (45 mL × 2). The organic lay was combined and washed with saturated $NaHCO_3$ (45 mL × 2) and H_2O (45 mL × 2) and dried over $NaSO_4$ for 4 h. The solvent was removed under reduced pressure and the residue was purified through column chromatography on silica gel (eluent ethyl acetate/petroleum ether, 3/7) to give **1b** as a white powder. Yield 54%; m.p. 105–107°C, ^{1}H NMR ($CDCl_3$, 600 MHz): δ 5.24–5.21 (1H, t, $J = 9.6$ Hz), 5.11–5.07 (1H, t, $J = 9.6$ Hz), 5.04–5.01 (1H, t, $J = 9.6$ Hz), 4.58–4.57 (1H, d, $J = 7.8$ Hz), 4.28–4.25 (1H, dd, $J = 12.4$, 5.0 Hz), 4.19–4.16 (2H, m), 2.09 (3H, s), 2.08 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (CDCl3,150 MHz): δ 170.8, 170.5, 169.6, 169.5, 101.1, 72.7, 72.0, 71.1, 69.9, 68.4, 61.9, 30.0, 20.9, 20.8, 20.7, 20.6; ESI-MS 477.1 $(M + Na)^+$.

General Method for the Synthesis of Methoxy or Benzyloxy Substituted (E,E)-4,6-bis(styryl)-2-Hydroxyl-Pyrimidines (3a–3d)

Compound **2a** (2.18 g, 15 mmol) and different substituted benzaldehyde (45 mmol) were dissolved in anhydrous ethyl alcohol (150 mL) in a round-bottomed flask (500 mL). Then 3 mL concentrated hydrochloric acid was added and the mixture refluxed at 100°C for 36 h. After completion of the reaction, the solvent was evaporated under reduced pressure. The saturated $NaHCO_3$ solution (250 mL) was added into the brown oil residue for desalting. After stirring at rt for 5 h, the resulting precipitate was filtered and washed with water and ether to obtain crude product and then purified by chromatography on silica gel (eluent, dichloromethane/methanol) to give **3a–d** as brown powder.

(*E*,*E*)-4,6-bis(3,4-Dimethoxystyryl)-2-hydroxyl-pyrimidine (**3a**) yield 70%; m.p. 255–257°C; ^{1}H NMR (DMSO-d_6, 600M): δ 11.55 (1H, s), 7.80 (1H, s), 7.77 (1H, s), 7.29 (2H, s), 7.20 (2H, d, $J = 7.74$), 7.04–7.02 (2H, d, $J = 7.92$),

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6.94–6.92 (2H, d, $J = 15.42$), 6.85 (1H, s), 3.84 (6H, s), 3.81 (6H, s); ^{13}C NMR (DMSO-d6, 150M): δ 151.09, 149.60, 138.78, 128.63, 122.66, 112.31, 110.39, 56.13 ppm; ESI-MS 421.2 $(M + H)^+$.

(*E*,*E*)-4,6-bis(3, 4, 5-Trimethoxystyryl)-2-hydroxyl-pyrimidine (**3b**) yield 65%; m.p. 222–224°C, ^{1}H NMR (DMSO-d_6, 600M): δ 11.71 (1H, s,), 7.79 (2H, d, $J = 16.5$Hz), 6.99 (7H, m), 3.85 (12H, s), 3.71 (6H, s) ppm; ^{13}C NMR (DMSO-d_6, 150M): δ 153.7, 139.6,139.0, 131.4, 107.6, 105.7, 67.2, 60.7, 56.5 ppm; ESI-MS 481.2 $(M + H)^+$.

(*E*,*E*)-4,6-bis(4-Methoxystyryl)-2-hydroxyl-pyrimidine (**3c**) yield 69%; m.p. 210–212°C, ^{1}H NMR (DMSO-d6, 600M): δ 7.81 (1H, s), 7.79 (1H, s), 7.63–7.62 (4H, d, $J = 8.22$), 7.03–7.02 (4H, d, $J = 8.28$), 6.89 (1H, s), 6.86 (2H, s), 3.81 (6H, s) ppm; ^{13}C NMR (DMSO-d_6, 150M): δ 161.26, 138.53, 130.02, 128.38, 115.12, 55.90 ppm; ESI-MS 361.2 $(M + H)^+$.

(*E*,*E*)-4,6-bis(4-(Benzyloxy)styryl)-2-hydroxyl-pyrimidine (**3d**) yield 66%; m.p. 215–217°C, ^{1}H NMR (DMSO-d_6, 600M), δ 11.62 (1H, s), 7.80 (1H, s), 7.78 (1H, s), 7.62–7.61 (4H, d, $J = 7.68$), 7.47–7.46 (4H, d, $J = 7.68$), 7.42–7.39 (4H, t, $J = 7.44$), 7.35–7.34 (2H, d, $J = 7.14$), 7.11–7.09 (4H, d, $J = 8.28$), 6.89 (1H, s), 6.87 (1H, s), 6.84 (1H, s), 5.17 (4H, s) ppm; ^{13}C NMR (DMSO-d_6, 150M): δ 160.31, 137.30, 129.99, 129.04, 128.52, 128.34, 115.94, 69.93 ppm; ESI-MS 513.2 $(M + H)^+$.

General Method for the Synthesis of Alkoxy Substituted (E,E)-4,6-bis(styryl)-2-O-(2,3,4,6,-tetra-O-acetyl)Glucopyranosyl-Pyrimidines (4a-4d) and (E,E)-4,6-bis(3,4-dimethoxystyryl)-2-(2-O-(2,3,4,6-tetra-O-acetyl)glucopyranosyl)Ethyloxgyl-Pyrimidine (4e)

A mixture of 10 mL H_2O and 10 mL $CHCl_3$ was stirred at 40°C and tetrabutyl ammonium bromide (484 mg 1.5 mmol) was added. Then a solution of K_2CO_3 (622 mg 4.5 mmol) and compounds **3a–d** (1.5 mmol) in 25 mL H_2O and 10 mL DMF was added to the mixture. After that, solution of compound **1a** or **1b** (2.0 mmol) in $CHCl_3$ (25 mL) was added dropwise at 40°C over a period of 30 min to the solution. The reaction mixture was allowed to stir overnight at 40°C. After completion of the reaction, the mixture was extracted with CH_2Cl_2 (50 mL × 3). The organic layer was combined and washed with H_2O (50 mL × 3) and dried over $MgSO_4$ for 4 h. Then the solution was removed under reduced pressure and purified by chromatography on silica gel (eluent, ethyl acetate/petroleum ether) to give product **4a–e** as yellow solid.

(*E*,*E*)-4,6-bis(3,4-Dimethoxystyryl)-2-*O*-(2,3,4,6,-tetra-O-acetyl)glucopyranosyl-pyrimidine (**4a**) yield 65.5%; m.p. 93–95°C; ^{1}H NMR ($CDCl_3$, 600M): δ 7.84 (1H, s), 7.82 (1H, s), 7.19 (1H, d, $J = 2.22$), 7.17 (1H, d, $J = 1.62$), 7.15

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(2H, d, $J = 1.62$), 6.99 (1H, s), 6.91–6.89 (3H, d, $J = 6.6$), 6.88 (1H, s), 6.36–6.35 (1H, d, $J = 8.22$), 5.45–5.42 (1H, t, $J = 9.36$), 5.39–5.36 (1H, t, $J = 8.79$), 5.23–5.20 (1H, t, $J = 9.6$), 4.21–4.20 (2H, t, $J = 3.75$), 4.05–4.02 (1H, m), 3.96 (6H, s), 3.93 (6H, s), 2.07 (3H, s), 2.05 (3H, s), 2.03 (3H, s), 1.94 (3H, s) ppm; ^{13}C NMR ($CDCl_3$, 150M): δ 170.8, 170.5, 169.5, 169.3, 165.4, 163.5, 150.6, 149.3, 137.4, 128.7, 123.3, 121.9, 112.1, 111.2, 109.7, 73.4, 72.4, 71.1, 68.6, 62.2, 56.0, 20.7 ppm HRMS (ESI) M/Z calcd. for $C_{38}H_{43}N_2O_{14}$ [M+H$^+$]: 751.2709, found 751.2708.

(*E*,*E*)-4,6-bis(3,4,5-Trimethoxystyryl)-2-*O*-(2,3,4,6,-tetra-O-acetyl)glucopyranosyl-pyrimidine (**4b**) Yield 56.3%; m.p. 108–110°C, ^{1}H NMR ($CDCl_3$, 600M): δ 7.83 (1H, s), 7.80 (1H, s), 7.03 (1H, s), 6.94 (1H, s), 6.92 (1H, s), 6.85 (4H, s), 6.37–6.36 (1H, d, $J = 8.22$), 5.46–5.43 (1H, t, $J = 9.36$), 5.39–5.36 (1H, t, $J = 8.28$), 5.23–5.20 (1H, t, $J = 9.9$), 4.21–4.20 (2H, t, $J = 2.22$), 4.06–4.03 (1H, m), 3.94 (12H, s), 3.90 (6H, s), 2.07 (3H, s), 2.06 (3H, s), 2.03 (3H, s), 1.96 (3H, s) ppm; ^{13}C NMR ($CDCl_3$, 150M): δ 170.8, 170.6, 169.5, 169.3, 165.3, 163.6, 153.6, 139.7, 137.7, 131.2, 124.7, 112.5, 105.0, 94.4, 73.3, 72.3, 71.0, 68.6, 62.2, 61.1, 56.3, 20.7 ppm; HRMS (ESI) M/Z calcd. for $C_{40}H_{47}N_2O_{16}$ [M+H$^+$]: 811.2920, found 811.2918.

(*E*,*E*)-4,6-bis(4-Methoxystyryl)-2-*O*-(2,3,4,6,-tetra-O-acetyl)glucopyranosyl-pyrimidine (4c) yield 50.2%; m.p. 225–227°C, ^{1}H NMR ($CDCl_3$, 600M): δ 7.86 (1H, s), 7.83 (1H, s), 7.56–7.54 (4H, d, $J = 8.82$), 6.94–6.93 (5H, t, $J = 4.41$), 6.90 (1H, s), 6.87 (1H, s), 6.35–6.33 (1H, d, $J = 8.28$), 5.44–5.41 (1H, t, $J = 9.36$), 5.39–5.36 (1H, t, $J = 8.79$), 5.22–5.19 (1H, t, $J = 9.63$), 4.23–4.19 (2H, m), 4.03–4.00 (1H, m), 3.85 (6H, s), 2.07 (3H, s), 2.05 (3H, s), 2.02 (3H, s), 1.93 (3H, s) ppm; ^{13}C NMR ($CDCl_3$, 150M): δ 170.8, 170.5, 169.5, 169.3, 165.5, 163.5, 160.9, 137.2, 129.3, 128.4, 123.1, 114.4, 112.3, 94.4, 73.6, 72.5, 71.0, 68.6, 62.2, 55.5, 20.7 ppm; HRMS (ESI) M/Z calcd. for $C_{36}H_{39}N_2O_{12}$ [M+H$^+$]: 691.2498, found 691.2495.

(*E*,*E*)-4,6-bis(4-(Benzyloxy)styryl)-2-*O*-(2,3,4,6,-tetra-O-acetyl)glucopyranosyl-pyrimidine (4d) yield 47.6%; m.p. 173–175°C, ^{1}H NMR ($CDCl_3$, 600M): δ 7.85 (1H, s), 7.83 (1H, s), 7.55–7.54 (4H, d, $J = 8.82$), 7.44–7.43 (4H, d, $J = 7.14$), 7.41–7.38 (4H, t, $J = 7.68$), 7.35–7.33 (4H, t, $J = 7.56$), 7.01–6.99 (4H, d, $J = 8.82$), 6.92 (1H, s), 6.89 (1H, s), 6.87 (1H, s), 6.34–6.33 (1H, d, $J = 8.28$), 5.44–5.41 (1H, t, $J = 9.33$), 5.39–5.6 (1H, t, $J = 8.82$), 5.22–5.19 (1H, t, $J = 9.63$), 5.10 (4H, s), 4.21–4.16 (2H, m), 4.02–3.99 (1H, m), 2.07 (3H, s), 2.05 (3H, s), 2.07 (3H, s), 1.93 (3H, s) ppm; ^{13}C NMR ($CDCl_3$, 150M): δ 170.8, 170.5, 169.5, 169.4, 165.5, 163.5, 160.1, 136.6, 129.3, 128.7, 128.7, 128.2, 127.5, 123.2, 115.3, 112.4, 73.6, 72.5, 71.0, 70.2, 62.2, 20.7 ppm; HRMS (ESI) M/Z calcd. for $C_{48}H_{47}N_2O_{12}$ [M+H$^+$]: 843.3124, found 843.3132.

(*E*,*E*)-4,6-bis(3,4-Dimethoxystyryl)-2-(2-O-(2,3,4,6-tetra-O-acetyl)glucopyranosyl)ethyloxgyl-pyrimidine (**4e**) yield 40.7%; m.p. 90–92°C, ^{1}H NMR ($CDCl_3$, 600M): δ 7.85 (1H, s), 7.82 (1H, s), 7.18–7.16 (2H, dd, $J = 2.19, 8.22$), 7.14 (2H, d, $J = 1.68$), 6.92 (1H, s), 6.90 (1H, s), 6.88 (2H, d, $J = 3.3$), 6.87

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(1H, s), 5.25–5.22 (1H, t, $J = 9.37$), 5.12–5.09 (1H, t, $J = 9.6$), 5.05–5.02 (1H, t, $J = 8.79$), 4.73–4.72 (1H,d, $J = 8.28$), 4.71–4.67 (1H, m), 4.61–4.58 (1H, m), 4.28–4.24 (2H, m), 4.13–4.10 (1H, dd, $J = 2.22, 12.12$), 4.07–4.03 (1H, m), 3.96 (6H, s), 3.93 (6H, s), 3.77–3.74 (1H, m), 2.07 (3H, s), 2.02 (3H, s), 2.01 (3H, s), 1.99 (3H, s) ppm; ^{13}C NMR ($CDCl_3$, 150M): δ 170.8, 170.4, 169.6, 165.2, 150.4, 149.3, 136.9, 128.8, 123.7, 121.8, 111.2, 109.5, 101.2, 72.9, 71.9, 71.3, 68.4, 68.2, 66.0, 62.0, 56.0, 20.8 ppm; HRMS (ESI) M/Z calcd. for $C_{40}H_{47}N_2O_{15}$ [M+H$^+$]: 795.2971, found 795.2980.

General Method for Synthesis of Methoxy or Benzyloxy Substituted (E,E)-4,6-bis(styryl)-2-O-Glucopyranosyl-Pyrimidines (5a–5d) and (E,E)-4,6-bis(3,4-dimethoxystyryl)-2-(2-O-glucopyranosyl) Ethyloxgyl-Pyrimidine (5e)

To a round-bottomed flask (100 mL), compound **4a–e** (0.5 mmol) was dissolved in 30 mL anhydrous CH_3OH and then 2 mL 0.5 M CH_3ONa/CH_3OH was added to the solution. The mixture was allowed to stir on at rt for 3 h. When the reaction was completed (monitored by TLC), 5g 732-type cation exchange resin was added to the reaction solution, and the mixture was stirred at rt for 5 min. Then the solvent was filtered and removed under reduced pressure. The crude products were recrystallized from cold methanol to give product **5a–e** as yellow solids.

(*E*,*E*)-4,6-bis(3,4-Dimethoxystyryl)-2-*O*-glucopyranosyl-pyrimidine (**5a**) yield 82.3%; m.p. 207–209°C, ^{1}H NMR (DMSO-d_6, 600M): δ 7.86 (1H, s), 7.83 (1H, s), 7.37 (2H, d, $J = 1.68$), 7.28 (1H, s), 7.26–7.24 (2H, dd, $J = 1.68, 8.82$), 7.16 (1H, s), 7.13 (1H, s), 7.02 (2H, d, $J = 8.28$), 5.93 (1H, d, $J = 7.68$), 5.42 (1H, d, $J = 5.46$), 5.16 (1H, d, $J = 4.38$), 5.07 (1H, d, $J = 5.52$), 4.60–4.58 (1H, t, $J = 5.79$), 3.85 (6H, s), 3.81 (6H, s), 3.70–3.68 (1H, m), 3.52–3.48 (1H, m), 3.40–3.72 (2H, m), 3.34–3.31 (1H, m), 3.24–3.21 (1H, m) ppm; ^{13}C NMR (DMSO-d_6, 150M): δ 165.62, 164.56, 150.82, 149.58, 137.45, 128.92, 124.16, 122.71, 112.24, 112.01, 110.53, 97.45, 78.22, 77.42, 73.43, 70.34, 61.24, 56.13 ppm; HRMS (ESI) M/Z calcd. for $C_{30}H_{35}N_2O_{10}$ [M+H$^+$]: 583.2286, found 583.2282.

(*E*,*E*)-4,6-bis(3,4,5-Trimethoxystyryl)-2-*O*-glucopyranosyl-pyrimidine (**5b**) yield 84.5%; m.p. 145–147°C, ^{1}H NMR (DMSO-d_6, 600M): δ 7.87 (1H, s), 7.84 (1H, s), 7.35 (1H, s), 7.25 (1H, s), 7.22 (1H, s), 7.07 (4H, s), 5.94–5.83 (1H, d, $J = 7.68$), 5.42–5.41 (1H, d, $J = 4.98$), 5.17–5.16 (1H, d, $J = 4.8$), 5.09–5.08 (1H, d, $J = 5.46$), 4.61–4.59 (1H, t, $J = 5.76$), 3.86 (12H, s), 3.70 (6H, s), 3.69–3.67 (1H, m), 3.51–3.47 (1H, m), 3.41–3.37 (2H, m), 3.33–3.30 (1H, m), 3.23–3.20 (1H, m) ppm; ^{13}C NMR (DMSO-d_6, 150M): δ 165.56, 164.63, 153.70, 139.36, 137.65, 131.69, 125.82, 112.23, 105.89, 97.48, 78.21, 77.41, 73.47, 70.36, 60.70, 56.58 ppm; HRMS (ESI) M/Z calcd. for $C_{32}H_{39}N_2O_{12}$ [M+H$^+$]: 643.2498, found 643.2495.

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(*E*,*E*)-4,6-bis(4-Methoxystyryl)-2-*O*-glucopyranosyl-pyrimidine (**5c**) yield 82.7%; m.p. 176–178°C, ^{1}H NMR (DMSO-d_6, 600M): δ 7.88 (1H, s), 7.85 (1H, s), 7.69–7.68 (4H, d, $J = 8.82$), 7.25 (1H, s), 7.11 (1H, s), 7.09 (1H, s), 7.02–7.00 (4H, d, $J = 8.82$), 5.91–5.89 (1H, d, $J = 7.68$), 5.42–5.41 (1H, d, $J = 5.52$), 5.16 (1H, d, $J = 4.92$), 5.07 (1H, d, $J = 5.52$), 4.59–4.57 (1H, t, $J = 6.06$), 3.81 (6H, s), 3.70–3.67 (1H, m), 3.51–3.48 (1H, m), 3.41–3.37 (2H, m), 3.34–3.32 (1H, m), 3.24–3.21 (1H, m) ppm; ^{13}C NMR (DMSO-d_6, 150M): δ 165.58, 164.50, 160.95, 137.01, 130.0, 128.68, 123.93, 114.98, 112.23, 97.51, 78.23, 77.43, 73.37, 70.33, 61.20, 55.86 ppm; HRMS (ESI) M/Z calcd. for $C_{28}H_{31}N_2O_8$ [M+H$^+$]: 523.2075, found 523.2062.

(*E*,*E*)-4,6-bis(4-(Benzyloxy)styryl)-2-*O*-glucopyranosyl-pyrimidine (**5d**) yield 84.0%; m.p. 212–214°C, ^{1}H NMR (DMSO-d_6, 600M): δ 7.87 (1H, s), 7.84 (1H, s), 7.69–7.68 (4H, d, $J = 8.76$), 7.48–7.47 (4H, d, $J = 7.14$), 7.42–7.39 (4H, t, $J = 7.44$), 7.35–7.33 (2H, t, $J = 7.44$), 7.24 (1H, s), 7.12 (1H, s), 7.09–7.08 (5H, d, $J = 8.76$), 5.90–5.89 (1H, d, $J = 7.68$), 5.42 (1H, d, $J = 4.98$), 5.17 (4H, s), 5.16 (1H, s), 5.07 (1H, d, $J = 4.98$), 4.59–4.57 (1H, t, $J = 6.06$), 3.70–3.67 (1H, m), 3.51–3.47 (1H, m), 3.40–3.37 (2H, m), 3.34–3.31 (1H, m), 3.24–3.20 (1H, m) ppm; ^{13}C NMR (DMSO-d_6, 150M): δ 165.56, 164.50, 160.04, 137.37, 136.96, 130.02, 129.04, 128.89, 128.49, 128.34, 124.05, 115.82, 112.31, 97.52, 78.25, 77.44, 73.37, 70.33, 69.90, 61.20 ppm; HRMS (ESI) M/Z calcd. for $C_{40}H_{39}N_2O_8$ [M+H$^+$]: 675.2701, found 675.2691.

(*E*,*E*)-4,6-bis(3,4-Dimethoxystyryl)-2-(2-O-glucopyranosyl)ethyloxgyl-pyrimidine (**5e**) yield 83.7%; m.p. 74–76°C, ^{1}H NMR (DMSO-d_6, 600M): δ 7.86 (1H, s), 7.84 (1H, s), 7.36 (2H, d, $J = 1.68$), 7.27–7.25 (2H, dd, $J = 2.16$, 8.76), 7.23 (1H, s), 7.14 (1H, s), 7.11 (1H, s), 7.02–7.01 (2H, d, $J = 8.28$), 5.10 (1H, d, $J = 5.1$), 5.00 (1H, d, $J = 4.92$), 4.96 (1H, d, $J = 4.98$), 4.57–4.55 (2H, t, $J = 6.03$), 4.27–4.26 (1H, d, $J = 7.68$), 4.18–4.14 (1H, m), 3.85 (6H, s), 3.80 (6H, s), 3.71–3.68 (1H, m), 3.64–3.63 (1H, d, $J = 5.46$), 3.49–3.43 (2H, m), 3.17–3.14 (2H, m), 3.09–3.07 (1H, m), 3.03–3.00 (1H, m) ppm; ^{13}C NMR (DMSO-d_6, 150M): δ 165.51, 165.21, 150.73, 149.55, 137.17, 128.93, 124.29, 122.40, 112.28, 110.66, 103.58, 77.53, 77.32, 74.00, 70.60, 67.46, 66.45, 61.68, 56.12 ppm; HRMS (ESI) M/Z calcd. for $C_{32}H_{39}N_2O_{11}$ [M+H$^+$]: 627.2548, found 627.2540.

Cytotoxicity Evaluation

The anticancer activity of synthesized compounds (**5a–e**) was evaluated in A549 and HL-60 cell lines in the Molecular Pharmacology Laboratory of our school. Cytotoxicity was determined using an MTT microplate assay and the results were expressed as the inhibition values of cell growth at the 10 μg/mL concentration of the compounds.

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MDR Reversal Activity Evaluation

The method of evaluating the MDR reversal activity of the compounds, such as cell culture, cell proliferation assay, cytotoxicity assay, and DOX accumulation assay, was carried out according to the reported procedures.[17]

CONCLUSIONS

In conclusion, five new compounds including five methoxy- or benzyloxy- substituted (*E*,*E*)-4,6-bis(styryl)-2-O-glucopyranosyl-pyrimidines and one (*E*,*E*)-4, 6-bis(3,4-dimethoxystyryl)-2-(2-O-glucopyranosyl)ethyloxgyl-pyrimidine were synthesized in four steps with total yields of 21.5% to 33.9%. The preliminary *in vitro* study demonstrated that most new compounds showed some inhibition effect against A549 and HL-60 cell lines at the concentration of 10 μg/mL. Meanwhile, the compounds **4a–e** were evaluated for their MDR reversal activity, and the results demonstrated compound **4a** and **4b** displayed high P-gp- and BCRP-modulating activity. The preliminary structure-activity relationship could be deduced and is of great significance for the finding of safe and effective MDR reversal agents.

In this paper, we have proposed a new strategy for the modification of curcuminoids with carbohydrate with no increase of the toxicity and found a novel type of MDR reversal agents that is very safe and effective. Further modification and evaluation of curcumin-carbohydrate conjugates are still needed to study their therapeutic efficacy and potential medicinal use.

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