# Synthesis, Characterization, Molecular Docking and Cytotoxicity Studies of Bagasse Xylem Ferulate-Acrylamide/Methyl Methacrylate Composite

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**ABSTRACT:** *BXFL (bagasse xylem ferulate)-g-AM (acrylamide)/MAA (methyl methacrylate) was synthesized based on bagasse xylan(BX)-g-AM/MAA in 1-Butyl-3-methylimidazolium chloride ([Bmim] Cl) ion solution with Ferulic Acid (FL) as esterification agent. The various factors that were investigated influenced carboxylic acid ester substitution degree ($DS_C$) of BXFL-g-AM/MAA. The structures of BXFL-g-AM/MAA were confirmed by IR, SEM and $^1H$ NMR analysis. The cytotoxicity of BXFL-g-AM/MAA was investigated against twelve kinds of protein through Surflex-Dock Mode of Sybyl-X 2.0 software, and on three kinds of human cancer cells line and the human normal cells line were detected by MTT assay. It was shown that the maximum $DS_C$ was 1.09 in ion solution, which was superior to $DS_C$ of conventional method in organic solvent. The results of MTT assay were consistent with docking results. BXFL-g-AM/MAA exhibited relative high cytotoxicity against human gastric cancer cells (MGC-803) line.*

**KEYWORDS:** *Bagasse Xylem ferulate-acrylamide/methyl methacrylate; Synthesis; Characterization; Cytotoxicity; Molecular docking.*

## INTRODUCTION

The tumor that is a common disease, frequently-occurring disease and serious threat to human health is one of the three major killers of human health [1]. Therefore exploring and developing new, cheap, safe and efficient anti-cancer becomes the newly expanded field. Extraction of anticancer active substances from natural products and improvement of its anticancer activity by structure modification will be the effective development the direction of new anticancer active medicine [2]. At present, anticancer components are extracted from plants to develop new anticancer drugs such as paclitaxel cancer drugs, camptothecin cancer drugs, teniposide, etoposid, vepesid, artesunate, salvicine, ginsenoside and elemene, etc [3-5]. And xylan as one of the most important natural products has received much attention because of its functional properties, such as antioxidant

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activity, anti-inflammatory effect, immunological regulation, anti-HIV activity, and cytotoxicity, etc [6-8]. Serial products of xylan like oats xylan sulfate, corn cob xylan sulfate, piperidine-N-sulfonic acid base xylan [7] were effective in anticoagulation and anti-AIDS. And some xylan grafted derivatives were effective in anti-cancer [8]. However, the defect of xylan that is low solubility in water, poor stability and difficult to process limits the application of xylan [9]. So xylan needs to be modified by chemical modification, which broadens its features and improves the additional value.

So far, chemical modification of xylan mainly contains esterification, etherification, oxidation, crosslinking and grafting, etc [10]. Not only were the functional defects of xylan improved, but its performance was enhanced after chemical modification. Particularly, the cytotoxic effect is especially striking through the modification of xylan [11]. Research shows that xylan derivatives can inhibit cancer cell proliferation, and most of them are graft copolymer. Besides, the ability to inhibit cancer cell proliferation is related to the molecular type and content of the grafting monomer [12-13]. The growth of length of branched chain and hydrophobic groups can exponentially improve pharmacological activity to inhibit tumor cancer cells [14]. If xylan-g-AM can be directed to tumor cells, it destroys the tumor cell's genetic material and achieves to inhibit cancer cell proliferation, such as human non-small cell lung cancer cells (NCI-H460), MGC-803 and human hepatoma cells (BEL-7407) line [15]. If MAA grafted onto xylan-g-AM increased length of branched chain and is higher cytotoxicity than xylan-g-AM. But a problem that limits its in-depth exploration in field of cytotoxicity is low cytotoxicity and weak selectivity because it can't identify specifically targeted cancer cells and there are a large number of hydroxyl groups that are not grafted (most grafting rate [16] is below 35%). To solve these problems, the esterified-grafted molecule of xylan has been designed and synthesized based on the xylan grafting copolymer. The esterification of xylan is divided into two types, direct esterification, and indirect esterification. Direct esterification, it is easy for xylan to react with esterification agents in organic solvents. Its activity and $DS_C$ are not high, because easily reactive esterification agents are generally low bioactive substances and medium strong acid and the xylan slightly dissolve in organic solvents [17] Indirect esterification, it is difficult for xylan to react with esterification agents in organic solvents. Esterification agents need to be modified by acyl chloride reaction. It can get high $DS_C$, but yields of acyl chloride reaction are very low, resulting in a large number of the waste of esterification agents, even the side effects of esterification agent [18]. Direct esterification and indirect esterifications are both solid and liquid reactions or solid and solid reactions in organic solvents.

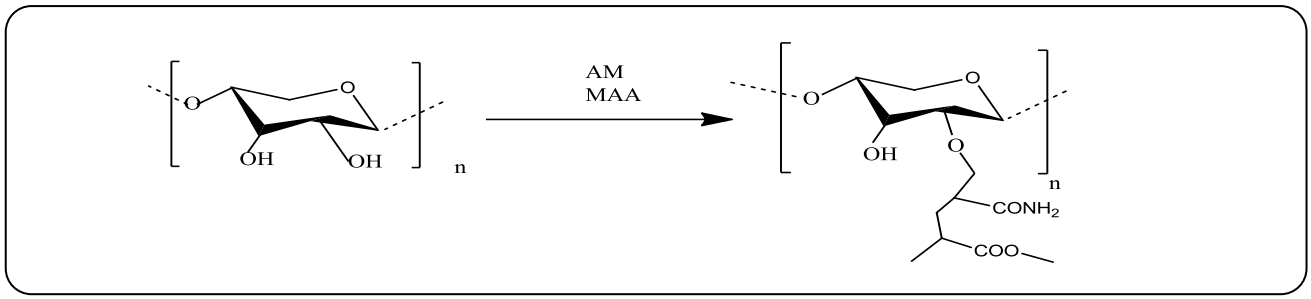
In this study, BXFL-g-AM/MAA was synthesized in [Bmim] Cl ion solution based on BX-g-AM/MAA and FL was introduced as esterification agent which can inhibit MGC-803 cell line [19]. [Bmim] Cl can dissolve BX of 50% [20]. It is a half liquid and liquid reaction that was easier than solid and liquid reaction. When the higher $DS_C$ is obtained through conditional optimization, its cytotoxicity was enhanced in MGC-803 line.

## EXPERIMENTAL SECTION

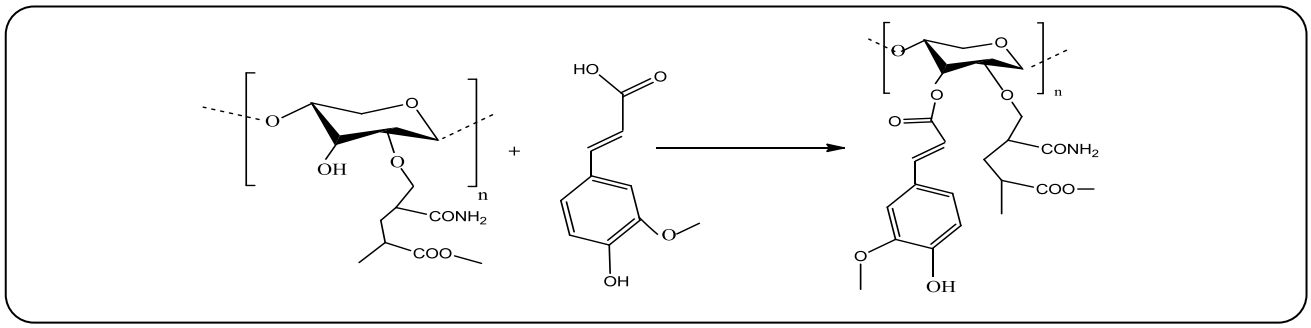
BX was provided by the Guilin institute of botany, China (purity of 80%, other impurities constituted of organic matter, cellulose, hemicellulose, lignin, and protein). Pyridine, *N,N'*-methylene bisacrylamide, MAA, ammonium persulfate, FL, AM, acetone, anhydrous ethanol, and p-toluene sulfonic acid were purchased from Xilong chemical plant, China. Hydrochloric acid was purchased from Hengyang Kaixin chemical reagent factor, China. Cyclohexane was purchased from national medicine group chemical reagent factor, China. DiMethyl sulfoxide-$d_6$ (D 99.9 %+0.05 % v/v TMS) was purchased from Cambridre Isotope Laboratories, USA. [Bmim] Cl was purchased from Shenzhen Tian Xudong technology co. LTD (China). FI-IR spectra of BX and BXFL-g-AM/MAA were recorded on FI-IR spectrometer (PERKIN-Elmer, USA) using potassium bromide (KBr) pellets. The morphology of sample was examined with JSM-6460LV scanning electron microscopy (JEOL, Japan). The thermal stability of sample was examined with SDT-Q600 Synchronous TGA/DSC analyzer (TA instrument, USA). *C* of cells line was tested from Guangxi Normal University for chemical and pharmaceutical molecular engineering laboratory.

### *Synthesis of BXFL-g-AM/MAA*

As described by Scheme 1 (BX-g-AM/MAA unit is diversity and complex, because AM and MAA irregularly




***Scheme 1: Synthesis of BX-g-AM/MAA.***




***Scheme 2: Synthesis of BXFL-g-AM/MAA.***

grafted onto BX), mixed monomer solution was obtained by 20mL of the mass fraction of 10% NaOH solution to neutralize to the mixture (according to the n (MAA): n (AM) =1: 1 proportion to joined a certain amount of solution in the flask) to pH=6. Mixed monomer solution (3mL), BX (1g), ammonium persulfate (0.1g) N,N'-methylene bisacrylamide (0.1g) and distilled water (50mL) were placed in the mouth flask(250mL) equipped with a dropping funnel, thermometer, condenser, and magnetic stirrer. The mixture was heated 50℃ for 4 h with stirring. At the end of the reaction, the mixture was filtered, washed, dried. In the way, BX-g-AM/MAA was obtained as powder in 25% grafting rate [16].

As described by Scheme 2 (BXFL-g-AM/MAA unit is more diverse and complex), BX-g-AM/MAA (5.6-28mmole, 0.1-0.5g/mL), [Bmim] Cl (0.13-0.52mol, 0.95g/L), FL (2.4-9.6mmole, 0.05-0.2g/mL) and p-toluene sulfonic acid (0.53-2.65mmole, 0.01-0.05g/mL) were placed in three mouth flasks (250mL) equipped with a thermometer, condenser, and magnetic stirrer in the nitrogen environment. The mixture was heated to 35-65 °C for 3.5~8.5 h with stirring. At the end of the reaction, it was added to the deionized water to precipitate out the solid. Then, it was stirred, filtered and dried. In this way, BXFL-g-AM/MAA was obtained as powder in 1.09 $DS_C$.

***$DS_C$ measurement***

$DS_C$ of BXFL-g-AM/MAA was measured through the principle of acid-base titration determination using a certain concentration of alkali solution and sample saponification reaction [18]. Then it was adjusted with neutrality by a certain concentration of acid. The last, $DS_C$ was calculated by the amount of consumption alkali. According to the principle of the above, the $DS_C$ was calculated by using the following equations:

$$w(\%) = \frac{(V_0 - V_1) \times 10^{-3} \times C_{HCl} \times 177}{m} \quad (1)$$

$$DS_C = \frac{286w}{177 - 176w} \quad (2)$$

Where *w* (%) is ester carbonyl mass fraction of BXFL-g-AM/MAA; $V_0$ is the volume of hydrochloric acid standard solution titration BX-g-AM/MAA (mL); $V_1$ is the volume of hydrochloric acid standard solution titration BXFL-g-AM/MAA (mL); $C_{HCl}$ is the concentration of hydrochloric acid standard solution (mol/L); *m* (g) is the mass of the sample of BXFL-g-AM/MAA;

***Inhibition rate (C) measurement***

*C* was measured by using the following equations [21]:

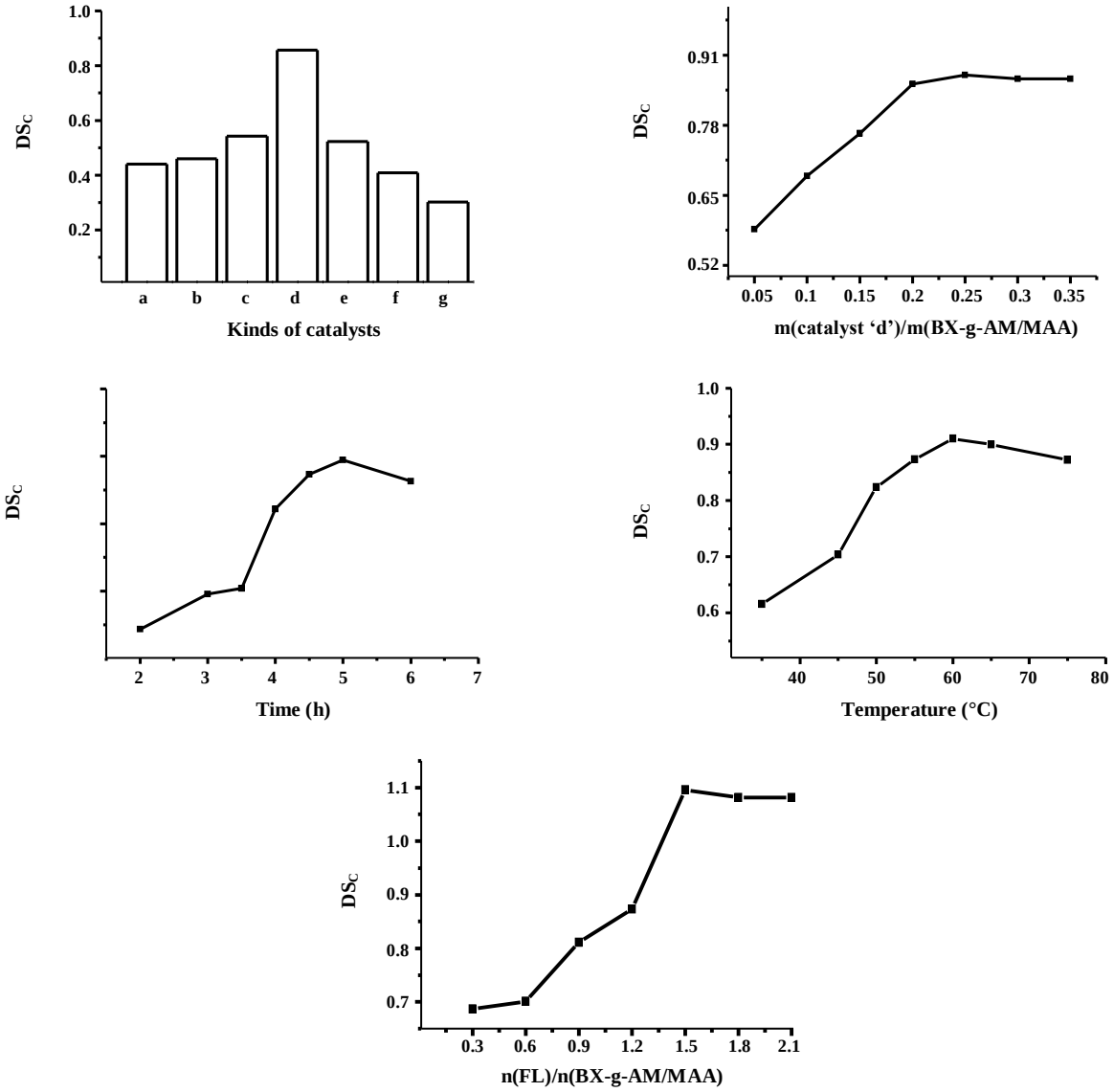



***Fig. 1: Effect of kinds of catalysts (A), m (catalyst'e')/m (BX-g-AM/MAA) (B), time (C), temperature (D) and mole ratio of FL and BX-g-AM/MAA (E) on $DS_C$.***

$$RGR\% = \frac{A}{B} \times 100\% \quad (3)$$

$$C = 1 - RGR\% \quad (4)$$

Where *A* is the experimental group absorbance (490-630nm); *B* is the control group absorbance (490-630nm); RGR % is cell relative increment rate; *C* is the inhibition rate.

## RESULTS AND DISCUSSION

### *Conditions optimization*

Defining optimum reaction conditions, we have studied the effect of various factors on $DS_C$ (Fig. 1): kinds of catalysts (sulfuric acid (a), phosphomolybdic acid hydrate (b), phosphotungstic acid (c), p-toluene sulfonic acid (d), tungstosilicic acid hydrate (e), pyridine (f), trimethylamine (g)), mass ratio of catalyst and BX-g-AM/MAA (0.05~0.35), esterification time (2~6 h), esterification temperature (35~75 °C), mole ratio of FL and BX-g-AM/MAA (0.3~2.1). [Bmim] Cl was solvent. BXFL-g-AM/MAA was obtained as a powder in 0.3~1.09. When conditions of the reaction are m (p-toluene sulfonic acid)/m (BX-g-AM/MAA)=0.25, time 5 h, temperature 60 °C, n (p-hydroxy salicylic acid)/n (BX-g-AM/MAA)=1.5, $DS_C$ was 1.09. It is superior to $DS_C$ of

conventional method in organic solvent and is a simple and quick way to get the medium and high $DS_C$.

***FT-IR spectrum***

It can be seen from Fig. 2, the comparison of absorption peaks revealed some changes in absorption, suggesting that BXFL-g-AM/MAA was successfully prepared. The broad band between 3000 and 3700 $cm^{-1}$ are assigned to O-H stretching. The absorption peak at 1719.82 $cm^{-1}$ is attributed to stretching vibration of O-C=O (xylan ferulic acid ester and MAA has ester bond overlap in the location of the 1719.82 $cm^{-1}$), the absorption peak at 689.54 and 1649.73 $cm^{-1}$ is attributed to stretching vibration of amide, the absorption peak at 1687.48 $cm^{-1}$ is attributed to stretching vibration of C=C, the peak at 1450.41$cm^{-1}$ has resulted from benzene ring frame vibration.

***SEM analysis***

Fig. 3a showed the morphology of the BX sample further revealed by SEM. The BX sample exhibits an oval and the surface of the complete. It is an amorphous state. Fig. 3b showed BXFL-g-AM/MAA sample exhibits that color is deeper than BX and surface structure have a great change.

***$^1$H NMR analysis***

To further confirm the structure of BXFL-g-AM/MAA, $^1$H-NMR spectroscopy was conducted on a sample slightly dissolved in DMSO-$d_6$, and the results are shown in Fig. 4, As can be seen, $^1$H NMR (500 MHz, DMSO) δ 7.21 (s, 1H), 6.84–6.80 (m, 1H), 6.67 (s, 1H), 5.39–5.30 (m, 2H), 5.12–5.06 (m, 1H), 5.00–4.95 (m, 1H), 4.28 (s, 1H), 3.88 (s, 2H), 3.52 (s, 3H), 3.26 (s, 2H), 3.17–3.13 (m, 1H), 3.06 (s, 1H), 2.59 (s, 1H), 2.34 (d, J=8.0 Hz, 1H), 2.10 (s, 3H), 1.97 (s, 1H), 1.64 – 1.59 (m, 1H), 1.47 (s, 2H), 0.87 (dd, J=16.5, 9.8 Hz, 3H). These results further confirm that BXFL-g-AM/MAA has been successfully synthesized.

***Simulation study***

Proteins were screened by keyword of Protein Databank such as gastric cancer, non-small cell lung cancer, and hepatoma. By keyword screening, there were four kinds of gastric cancer proteins, fourteen kinds of hepatoma proteins and fourteen kinds of non-small cell

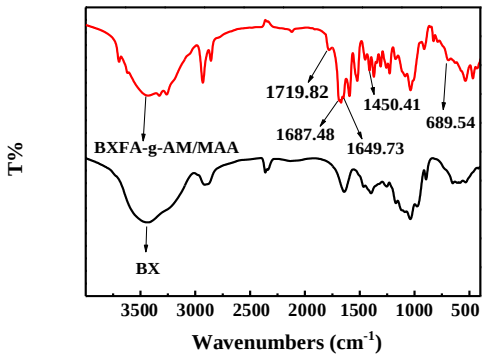



***Fig. 2: FT-IR spectra of the BX and BXFL-g-AM/MAA.***

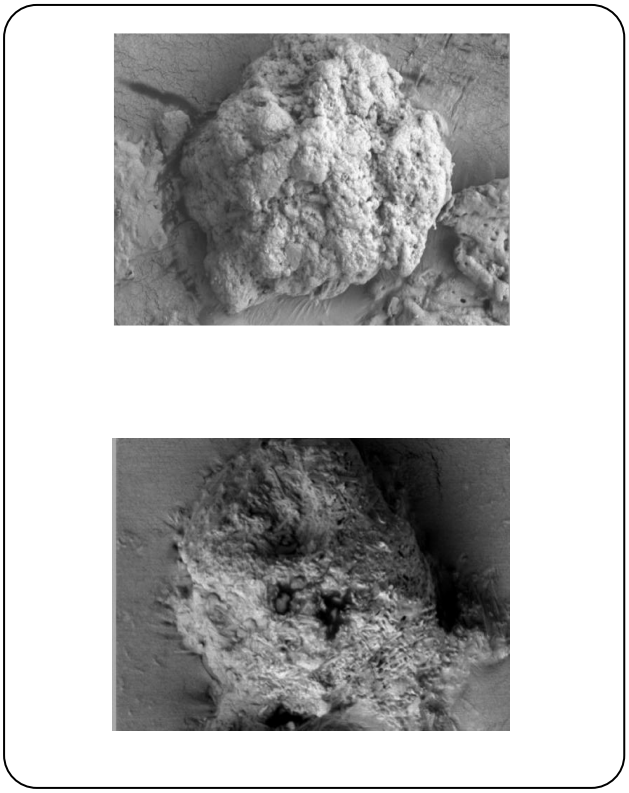


***Fig. 3: SEM image of BX (a) and BXFL-g-AM/MAA (b)***

lung cancer proteins except for only metal ligand protein. Then, four kinds of hepatoma (2NLU, 3EAE, 1N27, and 4UAI) and four kinds of non-small cell lung cancer proteins (2EB3, 4MKC, 5IWL, and 3HA6) were selected from different kinds of macromolecule respectively. Their ligands structures are similar to that of BXFL-g-AM/MAA, or no ligands. All gastric cancer proteins (4LN0, 4OUL, 4OUM, and 5J97) were selected from screening. Finally, twelve kinds of proteins

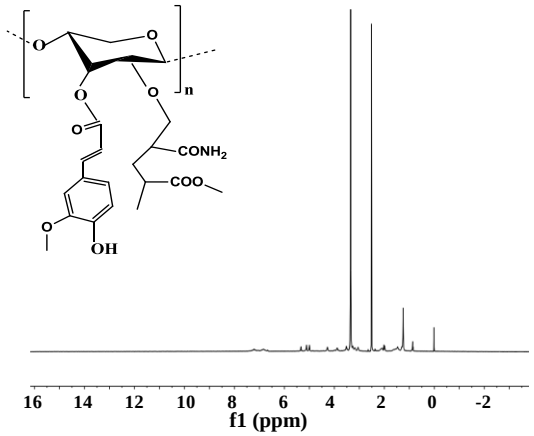



*Fig. 4: $^1$H NMR of BXFL-g-AM/MAA.*

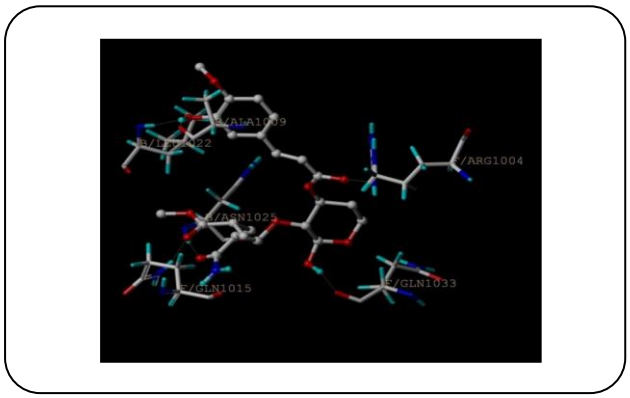



***Fig. 5: Hydrogen bonding interaction (yellow dotted line) of BXFL-g-AM/MAA unit (ball and stick mode) with amino acid residue (capped sticks) of 4OUL protein.***

were processed by extract ligand substructures, substructures removed, sidechain repaired, termini treated, and hydrogens added. And automatic mode generated SFXC file. BX unit, BX-g-AM/MAA and BXFL-g-AM/MAA unit were investigated against twelve kinds of proteins through the Surflex-Dock Mode of SYBYL-X 2.0 software [22], respectively. The highest Total score of each protein is chosen from docking analysis result, and C score and Global score are higher than or equal to 4.

BXFL-g-AM/MAA unit and 4OUL protein ranked first in docking analysis. The binding mode of BXFL-g-AM/MAA unit with amino acid residue of 4OUL protein is depicted on Fig. 5, moreover, red is O atom, white is C atom and cyan is H atom, blue is N atom. BXFL-g-AM/MAA unit formed hydrogen bonds contact with ALA1008, LEU1022, ASN1025, GLN1015, GLN1033, and ARG1004. And most hydrogen bonds come from AM, MAA, and FL. The more hydrogen bonds, the more stable the docking structure, the higher the Total score and the higher the cytotoxicity.

The glide scores of docking performance were listed on Tables 1-3 respectively. The result considers several comprehensive factors are evaluated the interaction between ligands and proteins. For analyzing the result of docking, three main parameters are considered: Total score, C score, and Global score. The total score is calculated by Crash, Polar, D score, PMF score, G score, Chem score, C score, and Global score. Total score>4 will indicate the docking success and it is possible to form hydrogen bonds between small molecules and proteins. The higher total score, the more stable the docking structure and the more hydrogen bonds. C score and Global score represent the degree of similarity between software model and experiment. When C score and Global score (5 is the highest) are higher than or equal to 4, docking results are very similar to the experimental values. According to the docking ratings of the situation, C score and Global score of the three substances are higher than or equal to 4, which indicates docking results are very similar to the experimental values. Most Total scores of BXFL-g-AM/MAA unit are higher than that of BX and BX-g-AM/MAA unit and are higher than 4. And most Total scores of BX-g-AM/MAA unit are higher than that of BX unit. FL, AM, and MAA may improve the cytotoxicity of BX on gastric cancer, non-small cell lung cancer, and hepatoma cell line. Most Total score of BX unit is lower than 4, indicating litter cytotoxicity on three kinds of human cancer cells line. The total score of each of gastric cancer proteins is little different from that of non-small cell lung cancer and hepatoma proteins in docking analysis of BX-g-AM/MAA unit. AM and MAA may improve cytotoxicity of BX, but not specific cytotoxicity. Most Total scores of each of gastric cancer proteins are significantly higher than that of non-small cell lung cancer and hepatoma proteins in docking analysis of BXFL-g-AM/MAA unit. The total score of each of non-small cell lung cancer proteins is a little different from that of hepatoma proteins. FL may be specific cytotoxicity on gastric cancer cells line.

### *MTT assay*

The cytotoxicity of BXFL-g-AM/MAA on three kinds of human cancer cells line and the human normal cells line were detected by MTT assay [21]. *C* is the inhibition

***Table 1: Glide score of protein receptors with BX unit.***

| PDB | Total score | Crash | Polar | D score | PMF score | G score | Chem score | C score | Global score |
|---|---|---|---|---|---|---|---|---|---|
| 4LN0 | 3.74 | -0.40 | 3.62 | -31.979 | -22.166 | -105.107 | -8.983 | 4 | 4 |
| 4OUL | 3.38 | -0.26 | 2.88 | -48.197 | -0.588 | -85.850 | -10.797 | 4 | 4 |
| 4OUM | 1.69 | -0.32 | 2.11 | -41.787 | -25.531 | -64.799 | -9.839 | 4 | 4 |
| 5J97 | 3.93 | -0.61 | 2.94 | -64.128 | -16.058 | -105.278 | -9.936 | 5 | 5 |
| 2NLU | 3.55 | -0.52 | 4.29 | --55.708 | -22.660 | -114.731 | -17.710 | 5 | 5 |
| 3EAE | 3.21 | -0.29 | 3.68 | -57.249 | -31.490 | -80.079 | -9.989 | 4 | 4 |
| 1N27 | 3.00 | -0.31 | 4.28 | -45.095 | -10.193 | -81.691 | -14.609 | 4 | 4 |
| 4UAI | 2.68 | -0.52 | 3.07 | -33.956 | -7.863 | -71.581 | -8.983 | 4 | 4 |
| 2EB3 | 2.85 | -0.90 | 2.67 | -48.294 | -0.841 | -92.464- | 10.871 | 4 | 4 |
| 4MKC | 2.56 | -0.48 | 2.29 | -44.322 | -13.328 | -75.632 | -12.889 | 4 | 4 |
| 5IWL | 3.41 | -0.27 | 5.31 | -40.598 | -42.819 | -107.536 | -12.751 | 4 | 4 |
| 3HA6 | 4.19 | -0.46 | 4.49 | -50.324 | -37.541 | -106.579 | -14.347 | 4 | 4 |

***Table 2: Glide score of protein receptors with BX-g-AM/MAA unit***

| PDB | Total score | Crash | Polar | D score | PMF score | G score | Chem score | C score | Global score |
|---|---|---|---|---|---|---|---|---|---|
| 4LN0 | 4.91 | -0.78 | 3.47 | -62.631 | -45.553 | -143.768 | -8.756 | 5 | 5 |
| 4OUL | 5.70 | -2.45 | 4.12 | -110.926 | -5.742 | -206.633 | -14.072 | 4 | 4 |
| 4OUM | 3.64 | -0.72 | 1.62 | -73.224 | -50.980 | -135.843 | -1.408 | 4 | 4 |
| 5J97 | 4.89 | -2.64 | 1.20 | -141.796 | -12.018 | -218.921 | -14.755 | 4 | 4 |
| 2NLU | 3.11 | -0.98 | 3.07 | -79.631 | -45.905 | -116.369 | -10.409 | 4 | 4 |
| 3EAE | 5.39 | -1.69 | 5.09 | -106.878 | -47.709 | -192.954 | -13.522 | 4 | 4 |
| 1N27 | 4.15 | -1.57 | 5.05 | -89.565 | -31.700 | -174.485 | -16.075 | 5 | 5 |
| 4UAI | 3.93 | -1.07 | 2.70 | -71.803 | -50.868 | -159.889 | -1.193 | 4 | 4 |
| 2EB3 | 5.43 | -1.70 | 5.85 | -103.420 | 68.712 | -125.107 | -17.980 | 4 | 4 |
| 4MKC | 4.66 | -2.48 | 3.43 | -112.059 | -31.695 | -157.366 | -9.469 | 4 | 4 |
| 5IWL | 4.32 | -2.57 | 4.35 | -114.284 | -67.213 | -174.777 | -9.529 | 4 | 4 |
| 3HA6 | 5.29 | -1.49 | 3.18 | -108.569 | -45.558 | -161.293 | -16.184 | 4 | 4 |

rate of the compound on cell lines. *C* of BX, BX-g-AM/MAA and BXFL-g-AM/MAA on NCI-H460, MGC-803, BEL-7407 and HC line which were listed on Tables 4-6 were detected by MTT assay in vitro, respectively. *C* of BX-g-AM/MAA on three cell lines is higher than that of BX. And there is no specific cytotoxicity. Grafting AM and MAA enhance cytotoxicity on three cell lines but do not increase specific cytotoxicity. *C* of BXFL-g-AM/MAA on three cell lines is higher than that of BX-g-AM/MAA and BX. and it exhibited relative high cytotoxicity against MGC-803 (IC50<50μg/mL) and a low inhibitory rate on HC line.

The particle of BX is composed of many chains of BX molecules in irregular form. AM, MAA and FL are mainly distributed on the surface of particle of BXFL-g-AM/MAA, because BX is a high polymer with particle and half dissolved condition and the hydroxyls of surface are more easily contacted with the AM, MAA and FL

***Table 3: Glide score of protein receptors with BXFL-g-AM/MAA unit.***

| PDB | Total score | Crash | Polar | D score | PMF score | G score | Chem score | C score | Global score |
|---|---|---|---|---|---|---|---|---|---|
| 4LN0 | 6.35 | -1.54 | 4.98 | -79.936 | -62.149 | -134.939 | -18.797 | 4 | 4 |
| 4OUL | 11.13 | -2.07 | 5.53 | -178.599 | -12.186 | -261.351 | -21.824 | 4 | 4 |
| 4OUM | 5.45 | -1.01 | 4.29 | -101.825 | -97.719 | -172.814 | -9.235 | 5 | 5 |
| 5J97 | 9.40 | -1.36 | 4.61 | -166.071 | -20.927 | -237.837 | -20.965 | 4 | 4 |
| 2NLU | 5.14 | -1.37 | 2.18 | -131.414 | -27.894 | -184.137 | -19.130 | 4 | 4 |
| 3EAE | 5.42 | -1.49 | 1.82 | -146.508 | -52.676 | -198.930 | -11.062 | 5 | 5 |
| 1N27 | 5.36 | -2.42 | 4.16 | -145.125 | -34.000 | -249.852 | -20.005 | 5 | 5 |
| 4UAI | 5.49 | -2.41 | 3.81 | -122.457 | -41.966 | -212.173 | -10.707 | 5 | 5 |
| 2EB3 | 5.78 | -1.91 | 5.04 | --157.407 | -57.508 | -218.064 | -23.487 | 5 | 5 |
| 4MKC | 5.88 | -2.06 | 3.71 | -145.246 | -28.680 | -192.183 | -15.484 | 5 | 5 |
| 5IWL | 6.04 | -2.50 | 3.43 | -177.324 | -79.497 | -235.398 | -11.437 | 4 | 4 |
| 3HA6 | 5.78 | -3.30 | 4.25 | -156.542 | -71.887 | -223.744 | -15.386 | 4 | 4 |

***Table 4: C (24h) of BX on NCI-H460, MGC80-3, BEL-7407 and HC line in different concentrations.***

| | 100μg/mL | 50μg/mL | 20μg/mL | 10μg/mL | 1μg/mL |
|---|---|---|---|---|---|
| NCI-H460 | 4.62±2.79 | 0.71±0.22 | -0.24±0.19 | -2.97±1.43 | -4.33±2.03 |
| MGC-803 | 2.02±0.57 | 0.24±0.08 | -0.15±0.13 | -2.99±1.11 | -3.27±1.61 |
| BEL-7407 | 1.07±0.71 | 1.18±0.34 | 0.35±0.26 | 0.47±0.29 | -0.45±0.31 |
| HC | 0.12±0.0.09 | -0.75±0.35 | -1.87±0.86 | -3.45±1.87 | -5.23±2.45 |

***Table 5: C (24h) of BX-g-AM/MAA on NCI-H460, MGC80-3, BEL-7407 and HC line in different concentrations.***

| | 100μg/mL | 50μg/mL | 20μg/mL | 10μg/mL | 1μg/mL |
|---|---|---|---|---|---|
| NCI-H460 | 11.04±4.51 | 3.46±1.13 | 0.20±0.15 | -0.97±0.23 | -2.39±1.27 |
| MGC-803 | 13.59±3.94 | 10.80±5.46 | 8.49±2.47 | 5.75±3.73 | 2.16±0.60 |
| BEL-7407 | 15.28±2.51 | 14.39±2.33 | 9.78±1.92 | 5.78±1.21 | 1.10±0.33 |
| HC | 5.69±3.01 | 2.19±1.13 | 0.29±0.09 | -1.29±0.41 | -3.38±1.12 |

***Table 6: C (24h) of BXFL-g-AM/MAA on NCI-H460, MGC80-3, BEL-7407 and HC line in different concentrations.***

| | 100μg/mL | 50μg/mL | 20μg/mL | 10μg/mL | 1μg/mL |
|---|---|---|---|---|---|
| NCI-H460 | 19.94±2.88 | 6.05±2.71 | 3.36±2.73 | 2.42±0.53 | 1.21±0.21 |
| MGC-803 | 68.58±5.94 | 53.12±5.21 | 37.41±4.14 | 25.34±3.26 | 8.32±1.36 |
| BEL-7407 | 18.46±3.09 | 13.30±1.22 | 10.01±4.72 | -0.28±0.68 | -2.59±1.12 |
| HC | 11.62±3.73 | 7.48±3.33 | 5.38±2.33 | 3.67±1.37 | 1.68±0.29 |

than internal hydroxyl. In MTT cytotoxic experiments, when the surface of the particle is slightly dissolved in the DMSO in different concentrations, AM MAA and FL of surface interact proteins with cells line to form hydrogen bonds. BXFL-g-AM/MAA expresses higher C of MGC-803 than BX-g-AM/MAA and FL (17), due to FL of surface to MGC-803 specific cytotoxic effects and AM and MAA of surface to cells line multiple cytotoxic. BXFL-g-AM/MAA expresses similar *C* of NCI-H460 and BEL-7407, due to only cytotoxic effect of AM and MAA and little specific cytotoxic effect of FL. BX and BX-g-AM/MAA expresses similar *C* of three cells line, indicating cytotoxic effects, but not specific cells. The results of MTT assay were consistent with docking results. All samples express low cytotoxicity on HC cells line, which shows BXFL-g-AM/MAA has low side effects and does not change the nature of natural products through esterification and grafting reaction.

## CONCLUSIONS

In the present study, BXFL-g-AM/MAA has been synthesized in [Bmim] Cl ion solution and can get a high 1.09 $DS_C$. It is superior to $DS_C$ of conventional method in organic solvent and a simple and highly efficient way to obtain the high $DS_C$. Ion solution as solvent provides some references for synthesizing xylan ester derivatives via the other kinds of xylans. Based on the IR, SEM and $^1$H NMR characterization, structure of BXFL-g-AM/MAA have a great change, and the color of BXFL-g-AM/MAA is deeper than BX. The results of MTT assay were consistent with docking results, indicating cytotoxic effect of AM and MAA on three kinds of cancer cells line and specific cytotoxic effect of FL on MGC-803 line. AM, MAA and FL are mainly distributed on the surface of BX and targeted cytotoxicity and cytotoxic substances interact proteins with of MGC-803 lines to form hydrogen bonds, providing a good structural feature for the toxicity of the directed enhancement of MGC-803 lines. As a natural product modification, BXFL-g-AM/MAA retains low side effects and highly effective cytotoxicity on MGC-803 lines. The results would have guided significance on the further theoretical research about BX as a carrier to develop other cytotoxic materials.

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