Design and Synthesis of Novel EGFR Inhibitors with L-Rhamnose–Benzoxazinone Hybrids

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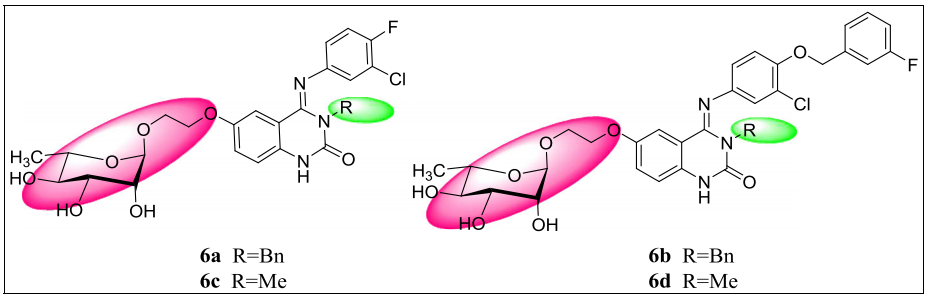
Additional Supporting Information may be found in the online version of this article.
Received March 13, 2015
DOI 10.1002/jhet.2463
Published online 00 Month 2015 in Wiley Online Library (wileyonlinelibrary.com).



Four L-rhamnose–benzoxazinone compounds as epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors were designed and synthesized. All structures of the compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, and high-resolution mass spectrometry. The inhibition activities of the target compounds for the EGFR tyrosine kinase activity *in vitro* were determined. Compounds **6a–d** displayed moderate activity in targeting EGFR.

INTRODUCTION

Receptor protein tyrosine kinases perform critical functions in the signal transduction pathways that regulate cell differentiation and proliferation [1]. The human epidermal growth factor receptor (HER) tyrosine kinase family consists of four structurally related cellular receptors: the epidermal growth factor receptor [epidermal growth factor receptor (EGFR), erbB1], erbB2/HER2, erbB3/HER3, and erbB4/HER4 [2]. Overexpression of EGFR has been associated with oncogenic activity, such as unregulated cell growth, proliferation, differentiation, and survival, and is also a marker for poor prognosis in many human cancers [3]. For this reason, EGFR has been an attractive target for the design of novel anticancer drugs. Inhibition of EGFR has been achieved using small-molecule inhibitors that interact at the adenosine-triphosphate-binding site [4]. Further development in this class has led to the discovery of gefitinib and lapatinib (Fig. 1), which have been approved for the chemotherapeutic treatment of patients with advanced non-small cell lung cancer [5–7].

In the previous years, various compounds, such as 4-anilinoquinazolines, 4-anilinopyrazolo[3,4-d]pyrimidines, benzoxazinone, and 4-anilinopyrroloquinazolines, have been reported as EGFR tyrosine kinase inhibitors [8]. Our group is active in the design and synthesis of benzoxazinone derivatives [9]. Benzoxazinone compounds with potential anticancer activity have certain similarities with the quinazoline ring [10]. Many studies have realized that modifying the quinazoline, with focus on the C-6 or C-7 position, is the reasonable strategy that developed many potent EGFR inhibitors [6]. H. R. Tsou and other workers reported that the core framework lied in a hydrophobic pocket [11]. A previous research by our group demonstrated that modifying benzoxazinone can improve its biological activity against EGFR [9]. However, most of the benzoxazinone derivatives exhibited poor solubility in water, which led to considerable difficulty in further research. To improve the water solubility and the biological activity, four novel benzoxazinone derivatives that incorporate water-solubilizing groups and bear different substituents at the N-3 position were designed and synthesized (Fig. 1).

Glycosides of physiologically active compounds (i.e., vitamin glycosides) have been reported [12]. Glycosylation allows water-insoluble and unstable organic compounds to be converted into their corresponding water-soluble and stable compounds, which could probably improve their bioavailability and pharmacological properties. In addition, the carbohydrate moiety may have an important function in their biological activities because saccharides contain a high density of functional groups. In this study, L-rhamnose ring was introduced into the target molecule. The effect on activity of different substituents at the 3-position, including methyl and benzyl, was then investigated. The resulting L-rhamnose–benzoxazinone compounds were evaluated *in vitro* using enzyme-linked immunosorbent assay.

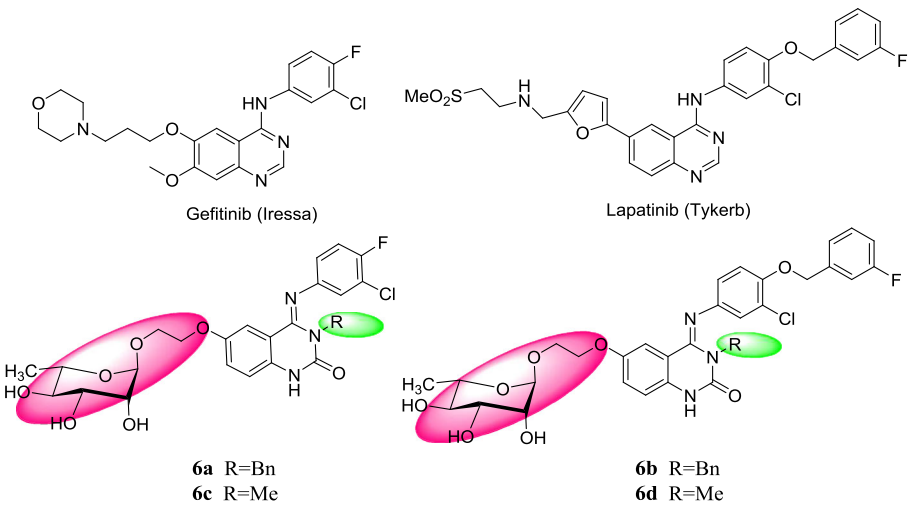
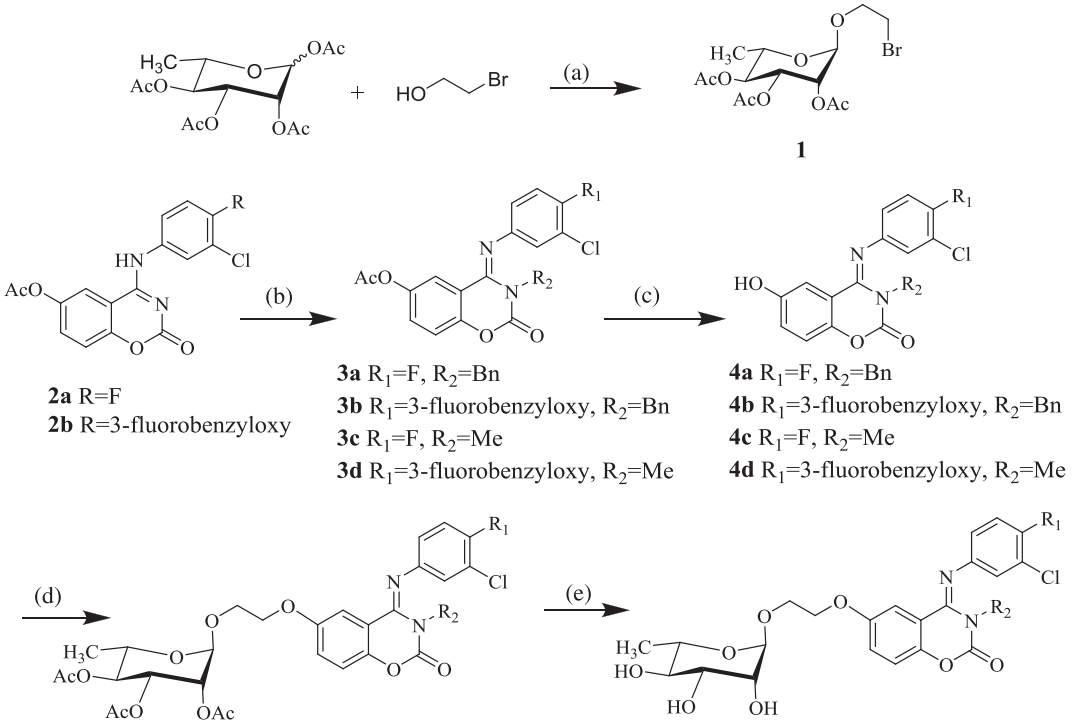


Figure 1. Structures of gefitinib, lapatinib, and compounds **6a–d**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RESULTS AND DISCUSSION

Synthesis of the target compounds 6a–d. 2,3,4,6-Tetra-*O*-acetyl-L-rhamnopyranosyl was synthesized as previously reported [13]. 1-*O*-bromoethy-2,3,4,6,-tetra-*O*-acetyl-β-L-rhamnopyranose (**1**) was synthesized as described in Experimental section. The key intermediates **2a**, **2b**, **3c**, **3d**, **4c**, and **4d** were obtained as described in the literature [9].

Scheme 1. Reagents and conditions: (a) BF_3Et_2O, CH_2Cl_2, 0°C, 20 h, 44%; (b) CH_3I, K_2CO_3, DMF, 70°C, 3 h, 69–78%; (c) concentrated ammonia liquor/MeOH, 91–96%; (d) $CHCl_3$, K_2CO_3, tetrabutylammonium bromide, DMF, 40°C, 4 h, 85–92%; (e) CH_3ONa/CH_3OH, 3 h, 66–95%.



The synthetic route of the target compounds is outlined in Scheme 1. First, benzylation was carried out through the reaction of compounds **2a** and **2b** with BnBr in dimethylformamide to afford **3a** and **3b**, respectively. Compounds **3a** and **3b** were then deacetylated by concentrated ammonia liquor (28%) in methanol to obtain **4a** and **4b**. Subsequently, intermediates **5a–d** were synthesized using tetrabutyl ammonium bromide as phase-transfer catalyst, which facilitated the migration of **4a–d** from the aqueous phase into the organic phase that contains **1** in chloroform. For the last step, compounds **5a–d** were deacetylated by a catalytic amount of CH_3ONa in methanol to obtain the target compounds **6a–d**, with yields of 66–95% after recrystallization in methanol. All the compounds were characterized by ^{1}H-NMR, ^{13}C-NMR, and high-resolution mass spectrometry (HRMS) spectrometry.

Evaluating the activity of compounds 3a–b, 4a–b, 5a–d, and 6a–d against epidermal growth factor receptor and the clogP values. The inhibition activities of compounds **3a–b**, **4a–b**, **5a–d**, and **6a–d** toward EGFR *in vitro* were determined by enzyme-linked immunosorbent assay [14] (Fig. 2). Our group evaluated the inhibition (%) of compounds **3c–d** and **4c–d**, as reported by Shaopeng Chen et al. [9] Previous studies have demonstrated that most synthesized benzoxazinone compounds showed low or moderate inhibition effect against EGFR *in vitro* at 10.0 μM concentration. Among the novel compounds, compounds **3a–d** and **4a–d** showed small inhibition rates (<40%). The activities of compounds **5a**, **5b**, and **5d** were better than that of **5c**, which is a hydroxyl-protected L-rhamnose–benzoxazinone compound. Compound **6a**, which had a benzyl substituted at the 3-position, displayed the best activity against EGFR (inhibition% =55.1%), whereas compounds **6b** and **6d** showed moderate inhibition effects against the isolated EGFR enzyme. The inhibition rate of the target compound **6c** against EGFR *in vitro* was 28.0% at 10.0 μM concentration. The four analogs exhibited lower EGFR inhibition activity compared with lapatinib.

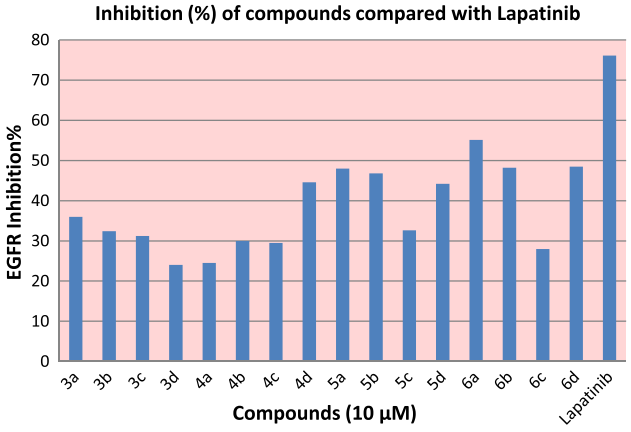


Figure 2. Epidermal growth factor receptor (EGFR) inhibition activities of the compounds (10 μM) and lapatinib. The data on inhibition (%) of compounds **3c–d** and **4c–d** were from Shaopeng Chen et al. [9]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

During the preparation of compounds, we found that the compounds **4a–d** could be easily dissolved in methanol but it is difficult to dissolve in water. In contrast, the compounds **6a–d** with sugar moiety are more soluble in water compared with methanol. Therefore, the clogP values of compounds **4a–d** and **6a–d** were calculated using SYBYL-X (Tripos Associates, St. Louis, MO). As shown in Table 1, compounds **6a–d**, with sugar moiety, have smaller clogP values than compounds **4a–d**, without the L-rhamnose moiety, again indicating that glycosylation improved the water solubility.

Binding mode analysis. To investigate whether the added saccharide ring can impact the bonding between compounds and EGFR, molecular docking was adopted to predict the binding modes of **6a-6d**. The docking simulations were carried out by means of the SYBYL-X 2.0 software, using the publicly available coordinate of the EGFR (PDB code: 1XKK). The compound **6a-6d** was docked into the EGFR model using standard default settings.

It can be seen from Figure 3A that **6a-6d** with sugar moiety and lapatinib adopts a completely opposite binding mode. The aniline groups of lapatinib lied in the hydrophobic pocket; in contrast, the aniline groups of **6a-6d** were positioned in the entrance of the binding pocket, while the sugar moiety lied in the same hydrophobic pocket. Figure 3B showed some main interactions (H-bond) between **6a** and the receptor. The oxygen atom of linker and the saccharide moiety of **6a** established H-bonds with Phe 856, Asp 855, and Thr 854, which may be the reasons why **6a-6d** adopts different binding mode. The H-bond could increase the binding affinities and the improving activity; however, the saccharide moiety as hydrophilic group was positioned in the hydrophobic pocket may be unfavorable to the activity.

Table 1

cLogP of compounds **4a–d**, **6a–d**, and lapatinib.

Compounds	cLogP
4a	5.07
4b	6.66
4c	3.30
4d	4.89
6a	4.41
6b	6.01
6c	2.65
6d	4.24
Lapatinib	5.90

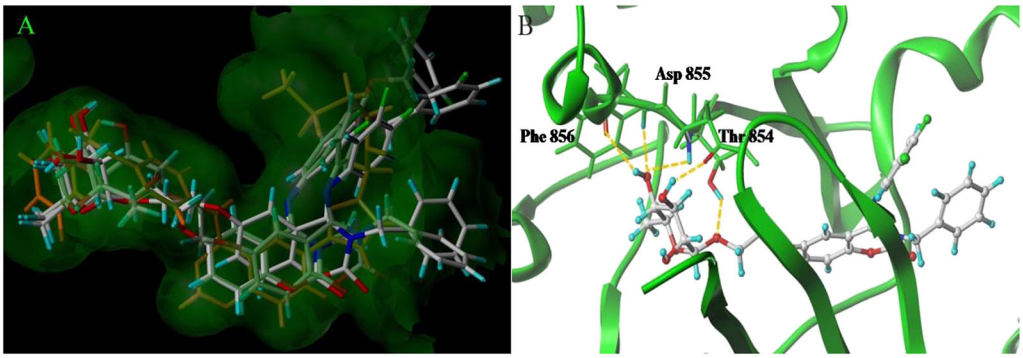


Figure 3. A. Overlay poses between Lapatinib (stick model with colored in orange) and **6a-6d** (stick model with colored by atom type). The binding site of the EGFR is shown in the transparent molecular surface colored in green. B. Low-energy docking model of the EGFR-TK/**6a** complex. The EGFR kinase is shown in a cartoon representation (green) with the inhibitor **6a** in a ball-and-stick representation with colored by atom type. H-bond interactions are shown as yellow dotted lines. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CONCLUSIONS

In conclusion, four target L-rhamnose–benzoxazinone compounds and eight novel intermediates were synthesized and characterized by NMR and HRMS spectrometry. The preliminary evaluation demonstrated that the compounds with L-rhamnose displayed moderate inhibition effect against EGFR at 10.0 μM concentration. The benzyl substituent at the 3-position was better than methyl. A strategy for the modification of benzoxazinone with carbohydrate was proposed, with the hope that it can be used to synthesize other inhibitors. Further modification and evaluation of L-rhamnose–benzoxazinone conjugates are still needed to study their therapeutic efficacy and potential medicinal use.

EXPERIMENTAL

General. All reagents used in the experiments were commercially available and purified in a conventional manner. Thin-layer chromatography was performed on Merck silica-gel 60 F_{254} plates (Darmstadt, Germany). Flash column chromatography was performed on silica gel (200–300 mesh, Qing dao, China). Melting points were measured on a WRX-1S melting-point apparatus (Taike Ltd., Beijing, China) and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were taken on JNM-ECP-600 spectrometer (Jeol Ltd, Tokyo, Japan) with tetramethylsilane (Me_4Si) as the internal standard. Mass spectra were recorded on a Q-TOF Global mass spectrometer (Micromass UK Ltd., Manchester, UK).

The data for compounds **2a**, **2b**, **3c**, **3d**, **4c**, and **4d** are the same as previously reported [9].

***Synthesis of 1-O*-bromoethy-2,3,4,6,-tetra-*O*-acetyl–β-D-L-rhamnose (1).** Full acety-L-rhamnose (0.33 g, 1.0 mmol) and 2-bromoethanol (1.1 mL, 1.2 mmol) were dissolved in dry CH_2Cl_2 (22 mL), stirring at 0°C under N_2 atmosphere. Then boron trifluoride ethyl ether (8.0 mL) was added dropwisely 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 root temperature. After completion of the reaction, the mixture was poured into ice water (37 mL) and extracted with CH_2Cl_2 (15 mL × 2). The organic lay was combined and washed with saturated $NaHCO_3$ (15 mL × 2) and H_2O (15 mL × 2) and dried over $MgSO_4$ 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, 1/3) to give the compound 1 as a white powder. (44% yield); mp 69–71°C; ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 5.31-5.29 (dd, J = 10.1, 3.2 Hz, 1H), 5.27-5.26 (m, 1H), 5.10-5.08 (t, J = 10.1 Hz, 1H), 4.79-4.78 (d, J = 1.4 Hz, 1H), 4.00-3.96 (m, 2H), 3.86-3.83 (m, 1H), 3.51-3.49 (t, J = 5.9 Hz, 2H), 2.16 (s, 3H, OAc), 2.06 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.24-1.22 (d, J = 5.9 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 170.3, 170.2, 170.1, 97.6, 71.0, 69.8, 69.1, 68.2, 66.9, 29.8, 21.0, 20.9, 20.8, 17.4; ESI-MS 419.0 $(M+Na)^+$.

4-(3-chloro-4-fluorophenylimino)-3-benzyl-2-oxo-3,4-dihydro-1, 3-benzoxazine-6-yl acetate (3a). A suspension of **2a** (500 mg, 1.43 mmol) and a hydrous potassium carbonate (400 mg, 2.89 mmol) in dry DMF (40 mL) was stirred at 40°C for 20 min; then BnBr (0.23 mL) was added, and the mixture was stirred at 70°C for 8 h under a N_2 atmosphere. After completion, the solvent was removed under reduced pressure and then purified by chromatography on silica gel to give **3a** (504 mg, 83% yield) as a yellow powder. mp 136–138°C; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 7.48-7.38 (overlap, 5H, ArH), 7.34-7.31 (t, J = 7.1 Hz, 2H, ArH), 7.26-7.23 (t, J = 7.0 Hz, 1H, ArH), 7.04-7.03 (dd, J = 6.6, 2.2 Hz, 1H, ArH), 6.82-6.80 (m, 1H, ArH), 6.70 (s, 1H, ArH), 5.24 (s, 2H), 2.15 (s, 3H, OAc); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 169.3, 154.9 (d, J = 180 Hz), 153.3, 149.3, 148.0, 146.1, 145.6, 144.0, 137.0, 128.8, 128.5, 128.0, 127.6, 121.1 (d, J = 36 Hz), 120.9, 120.7, 120.6, 119.8 (d, J = 53 Hz), 119.2, 118.5, 118.4, 47.5, 21.1; ESI-MS 439.0 $(M+H)^+$; HRMS (ESI): *Anal*. Calcd. for $C_{23}H_{17}N_2O_4FCl^+$, 439.0861. Found: 439.0846.

4-(3-chloro-4-(3-fluorobenzyloxy)phenylimino)-3-benzyl-2-oxo-3,4-dihydro-1,3-benzoxazine-6-yl acetate (3b). The same method that was used in getting **3a** was adopted to prepare **3b**. (80% yield); mp 114–116°C; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 7.48-7.40 (overlap, 5H, ArH), 7.34-7.30 (overlap, 4H, ArH), 7.26-7.24 (t, J = 7.7 Hz, 1H, ArH), 7.21-7.20 (d, J = 8.8 Hz, 1H, ArH), 7.18-7.16 (dd, J = 7.8, 2.1 Hz, 1H, ArH), 6.94-6.93 (d, J = 2.8 Hz, 1H, ArH), 6.76-6.74 (dd, J = 8.8, 2.2 Hz, 1H, ArH), 6.72 (br s, 1H, ArH), 5.24 (s, 2H), 5.22 (s, 2H), 2.13 (s, 3H, −OAc); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 169.3, 163.6, 162.0, 150.1, 149.2, 148.1, 146.1, 142.6, 140.3, 137.1, 131.1, 131.0, 128.8, 128.5, 128.0, 127.6, 123.7, 123.5, 120.8, 120.7, 119.1 (d, J = 20 Hz), 118.9, 116.7, 115.3, 115.2 (d, J = 21 Hz), 114.5, 114.4, 70.3, 47.3, 21.1; ESI-MS 545.1 $(M+H)^+$; HRMS (ESI): *Anal*. Calcd. for $C_{30}H_{23}N_2O_5FCl^+$, 545.1280. Found: 545.1263.

4-(3-chloro-4-fluorophenylimino)-6-hydroxy-3-benzyl-3,4-dihydro-1,3-benzoxazine-2-one (4a). Compound **3a** (381 mg, 0.87 mmol) was dissolved in methanol (30 mL), and then concentrated ammonia liquor (28%, 0.5 mL) was added. The reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, the solvent was evaporated under reduced pressure. The yellow precipitate formed was filtered and washed with diethylether (50 mL) to give **4a**. (94% yield); mp 215–217°C; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 9.77 (br s, ArOH), 7.41-7.38 (overlap, 3H, ArH), 7.34-7.31 (t, J = 7.1 Hz, 2H, ArH), 7.25-7.24 (t, J = 8.8 Hz, 1H, ArH), 7.23 (s, 1H, ArH), 7.03-7.01 (d, J = 9.4, 2.8 Hz, 2H, ArH), 6.77-6.76 (m, 1H, ArH), 6.44-6.43 (d, J = 2.8 Hz, 1H, ArH), 5.23 (s, 2H); ^{13}C-NMR (DMSO-d_6 150 MHz): δ 153.8 (d, J = 180 Hz), 148.3, 145.9, 144.5, 144.2, 143.0, 137.3, 128.8, 128.6, 128.0, 127.6, 127.1, 127.0, 122.5 (d, J = 36 Hz), 120.9, 119.5, 118.9 (d, J = 54 Hz), 118.1, 113.2, 112.5, 56.6; ESI-MS 397.1 $(M+H)^+$; HRMS(ESI): *Anal*. Calcd. for $C_{21}H_{15}N_2O_3FCl^+$, 397.0755. Found: 397.0755.

4-(3-chloro-4-(3-fluorobenzyloxy)phenylimino)-6-hydroxy-3-benzyl-3,4-dihydro-1,3-benzoxazine-2-one (4b). The same method that was used in getting **4a** was adopted to prepare **4b**. (97.5% yield); mp 189–191°C; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 9.74 (br s, 1H, ArOH), 7.47-7.45 (t, J = 6.1 Hz, 1H, ArH), 7.39-7.38 (d, J = 7.7 Hz, 2H, ArH), 7.34-7.31 (t, J = 7.7 Hz, 4H, ArH), 7.26-7.24 (t, J = 7.1 Hz, 1H, ArH), 7.22-7.21 (d, J = 6.6 Hz, 2H, ArH), 7.20-7.16 (dt, J = 8.8, 2.3 Hz, 1H, ArH), 7.03-7.01 (dd, J = 8.8, 2.8 Hz, 1H, ArH), 6.92-6.91 (d, J = 2.8 Hz, 1H, ArH), 6.72-6.71 (dd, J = 8.8, 2.7 Hz, 1H, ArH), 6.52-6.51 (d, J = 2.8 Hz, 1H, ArH), 5.24 (s, 2H), 5.22 (s, 2H); ^{13}C-NMR (DMSO-d_6, 150 MHz): δ 163.6, 161.9, 153.7, 149.9, 148.4, 144.5, 143.8, 142.9, 140.2, 137.4, 131.1, 131.0, 128.8, 128.0, 127.5, 123.8, 123.2, 122.2, 120.6 (d, J = 20 Hz), 118.8, 116.3, 115.3, 114.6, 114.5 (d, J = 21 Hz), 113.4, 112.7, 70.1, 47.1; ESI-MS 503.1 $(M+H)^+$; HRMS (ESI): *Anal*. Calcd. for $C_{28}H_{21}N_2O_4FCl^+$, 503.1174. Found: 503.1192.

(2R,3R,4R,5S,6S)-2-(2-(((Z)-3-benzyl-4-((3-chloro-4-fluorophenyl)imino)-2-oxo-3,4-dihydro-2H-benzo[e][1,3]oxazin-6-yl)oxy)ethoxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl triacetate (5a). A mixture of 10 mL H2O and 10 mL CHCl3 was stirred at 40°C, and tetrabutylammonium bromide (484 mg, 1.5 mmol) was added. Then a solution of K_2CO_3 (622 mg, 4.5 mmol) and compound **4a** (1.5 mmol) in 25 mL H_2O and 10 mL DMF was added to the mixture. After that, a solution of compound **1** (2.0 mmol) in $CHCl_3$ (25 mL) was added dropwisely 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 to give product **5a**. mp 55–58°C; ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 7.52-7.51 (d, J = 6.8 Hz, 2H, ArH), 7.34-7.32 (t, J = 7.8 Hz, 2H, ArH), 7.30-7.29 (d, J = 7.3 Hz, 1H, ArH), 7.20-7.16 (q, J = 8.7 Hz, 2H, ArH), 7.08-7.06 (dd, J = 8.5, 4.4 Hz, 1H, ArH), 6.88-6.87 (dd, J = 6.4, 2.8 Hz, 1H, ArH), 6.68-6.66 (m, 1H, ArH), 6.58-6.57 (d, J = 2.8 Hz, 1H, ArH), 5.38 (s, 2H), 5.19-5.18 (d, J = 1.9 Hz, 1H), 5.17-5.15 (m, 1H), 5.73-5.72 (d, J = 1.4 Hz, 1H), 4.24-4.23 (m, 1H), 3.81-3.79 (m, 2H), 3.63-3.62 (m, 3H), 2.15 (s, 3H, −OAc), 2.05 (s, 3H, −OAc), 2.00 (s, 3H, −OAc), 1.20-1.19 (d, J = 6.4 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 170.2, 170.1, 170.0, 154.4, 154.3 (d, J = 180 Hz), 148.5, 145.8, 145.3, 143.3, 136.5, 129.1, 128.5, 127.7, 123.2, 121.9 (d, J = 36 Hz), 120.8, 118.8, 118.6 (d, J = 54 Hz), 118.5, 117.7, 117.6, 111.9, 111.0, 97.6, 71.1, 69.7, 69.0, 66.9, 66.6, 66.0, 63.2, 21.0, 20.9, 20.8, 17.5; ESI-MS 713.2 $(M+H)^+$; HRMS (ESI): *Anal*. Calcd. for $C_{35}H_{35}N_2O_{11}FCl^+$, 713.1913. Found: 713.1932.

(2R,3R,4R,5S,6S)-2-(2-(((Z)-3-benzyl-4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)imino)-2-oxo-3,4-dihydro-2H-benzo[e][1,3]oxazin-6-yl)oxy)ethoxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl triacetate (5b). The same method that was used in getting **5a** was adopted to prepare **5b**. (85% yield); mp 65–68°C; ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 7.52-7.51 (d, J = 7.3 Hz, 2H, ArH), 7.38-7.36 (q, J = 7.7 Hz, 1H, ArH), 7.34-7.31 (t, J = 7.3 Hz, 2H, ArH), 7.28-7.27 (d, J = 7.3 Hz, 1H, ArH), 7.25-7.24 (d, J = 7.8 Hz, 1H, ArH), 7.22-7.21 (d, J = 9.1 Hz, 1H, ArH), 7.15-7.13 (d, J = 9.1 Hz, 1H, ArH), 7.06-7.03 (overlap, 2H, ArH), 7.00-6.98 (d, J = 8.7 Hz, 1H, ArH), 6.92-6.91 (d, J = 2.7 Hz, 1H, ArH), 6.64-6.63 (t, J = 2.3 Hz, 2H), 5.38 (s, 2H), 5.18-5.17 (t, J = 1.9 Hz, 1H), 5.15 (s, 2H), 5.13-5.12 (d, J = 3.7 Hz, 1H), 5.04-5.02 (t, J = 10.1 Hz, 1H), 4.70 (s, 1H), 3.80-3.78 (m, 1H), 3.74-3.70 (m, 1H), 3.59-3.56 (m, 3H), 2.13 (s, 3H, −OAc), 2.03 (s, 3H, −OAc), 1.96 (s, 3H, −OAc), 1.15-1.14 (d, J = 6.4 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 170.2, 170.1, 170.0, 154.3, 150.0, 148.6, 145.8, 143.1, 139.3, 136.7, 130.4, 129.1, 129.0, 128.7, 128.5, 128.4, 127.7, 124.7, 123.1,

122.5, 121.1, 118.7, 118.1, 116.0, 115.2, 115.0, 114.1, 114.0, 111.0, 97.6, 71.0, 70.9, 69.7, 69.0, 66.9, 66.5, 66.0, 47.1, 21.0, 20.9, 20.8, 17.4; ESI-MS 819.3 $(M+H)^+$; HRMS (ESI): *Anal*. Calcd. for $C_{42}H_{41}N_2O_{12}FCl^+$, 819.2332. Found: 819.2347.

(2R,3R,4R,5S,6S)-2-(2-(((Z)-4-((3-chloro-4-fluorophenyl)imino)-3-methyl-2-oxo-3,4-dihydro-2H-benzo[e][1,3]oxazin-6-yl)oxy)ethoxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl triacetate (5c). The same method that was used in getting **5a** was adopted to prepare **5c**. (92% yield); mp 61–64°C; ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 7.22-7.19 (t, J = 8.7 Hz, 1H, ArH), 7.18-7.16 (d, J = 8.7 Hz, 1H, ArH), 7.09-7.07 (dd, J = 8.7, 2.8 Hz, 1H, ArH), 6.97-6.96 (dd, J = 6.4, 2.8 Hz, 1H, ArH), 6.77-6.75 (m, 1H, ArH), 6.59 (br s, 1H, ArH), 5.20-5.19 (dd, J = 3.2, 1.4 Hz, 1H), 5.16-5.14 (dd, J = 10.1, 2.8 Hz, 1H), 5.06-5.03 (t, J = 10.1 Hz, 1H), 4.74-4.73 (d, J = 1.4 Hz, 1H), 3.85-3.81 (m, 2H), 3.65 (br s, 3H), 3.54 (br s, 3H), 2.15 (s, 3H, −OAc), 2.05 (s, 3H, −OAc), 1.98 (s, 3H, −OAc), 1.20-1.19 (d, J = 6.4 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 170.2, 170.1, 170.0, 155.2, 154.5, 148.5, 145.8, 145.6, 123.1, 123.0, 121.2, 121.1 (d, J = 36 Hz), 118.9, 118.7, 117.7, 117.5, 110.9, 97.6, 71.0, 69.7, 69.0, 66.9, 66.6, 66.0, 63.1, 21.0, 20.9, 20.8, 17.4; ESI-MS 637.1 $(M+H)^+$; HRMS (ESI): *Anal*. Calcd. for $C_{29}H_{31}N_2O_{11}FCl^+$, 637.1600. Found: 637.1621.

(2R,3R,4R,5S,6S)-2-(2-(((Z)-4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)imino)-3-methyl-2-oxo-3,4-dihydro-2H-benzo[e][1,3]oxazin-6-yl)oxy)ethoxy)-6-methyltetrahydro-2H-pyran-3,4,5-triyl triacetate (5d). The same method that was used in getting **5a** was adopted to prepare **5d**. (88% yield); mp 64–67°C; ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 7.38-7.36 (m, 1H, ArH), 7.37-7.25 (d, J = 10.5 Hz, 1H, ArH), 7.24-7.23 (d, J = 7.8 Hz, 1H, ArH), 7.16-7.14 (d, J = 9.1 Hz, 1H, ArH), 7.07-7.05 (d, J = 9.2, 2.8 Hz, 1H, ArH), 7.03-7.00 (overlap, 3H, ArH), 6.72-6.70 (dd, J = 8.3, 2.3 Hz, 1H), 6.65 (br s, 1H), 5.18 (s, 1H), 5.16 (s, 2H), 5.14-5.12 (dd, J = 10.1, 3.7 Hz, 1H), 5.04-5.01 (t, J = 9.6 Hz, 1H), 4.71 (s, 1H), 3.81-3.76 (m, 2H), 3.60 (br s, 3H), 3.53 (br s, 3H), 2.13 (s, 3H, −OAc), 2.04 (s, 3H, −OAc), 1.97 (s, 3H, −OAc), 1.16-1.15 (d, J = 6.4 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 150 MHz): δ 170.2, 170.1, 170.0, 150.1, 148.6, 145.7, 143.3, 139.3, 139.2, 130.4, 130.3, 124.7, 123.0, 122.5, 121.4, 118.6, 118.3, 115.9, 115.2, 115.0, 114.1, 114.0, 110.8, 97.6, 71.0, 70.8, 69.7, 69.0, 67.7, 66.9, 66.5, 66.1, 21.0, 20.9, 20.8, 17.4; ESI-MS 743.2 $(M+H)^+$; HRMS (ESI): *Anal*. Calcd. $C_{36}H_{37}N_2O_{12}FCl^+$, 743.2019. Found: 743.2038.

(Z)-3-benzyl-4-((3-chloro-4-fluorophenyl)imino)-6-(2-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)ethoxy)-3,4-dihydro-2H-benzo[e][1,3]oxazin-2-one (6a). To a round-bottomed flask (100 mL), compound **5a** (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 at root temperature for 3 h. When the reaction was completed (monitored by thin-layer chromatography), 5 g 732-type cation exchange resin was added to the reaction solution, and the mixture was stirred at root temperature for 5 min. Then the solvent was filtered and removed under reduced pressure. The crude products were recrystallized from cold ethanol to give product **6a**. (85% yield); mp 71–73°C; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 7.45-7.40 (overlap, 3H, ArH), 7.35-7.32 (t, J = 7.3 Hz, 3H, ArH), 7.27-7.23 (overlap, 2H, ArH), 7.09-7.08 (dd, J = 6.9, 1.4 Hz, 1H, ArH), 6.85-6.84 (m, 1H, ArH), 6.50 (s, 1H, ArH), 5.26 (s, 2H), 4.76 (br s, 3H), 4.60-4.54 (m, 2H), 3.71-3.68 (s, 2H), 3.64-3.60 (m, 2H), 3.58 (s, 1H), 3.19-3.17 (m, 2H), 1.09-1.08 (d, J = 6.4 Hz, 3H); ^{13}C-NMR (DMSO-d_6, 600 MHz): δ 154.8, 153.8 (d, J = 180 Hz), 148.2, 146.1, 145.8, 144.4, 137.2, 128.9, 128.8, 128.0, 127.6, 122.7, 121.0, 119.9 (d, J = 36 Hz), 119.2, 118.4 (d, J = 54 Hz), 112.5, 111.8, 100.7, 100.6, 72.4, 71.1, 70.9, 69.0, 67.7, 65.0, 60.7, 18.4; ESI-MS 587.1 $(M+H)^+$; HRMS (ESI): *Anal*. Calcd. for $C_{29}H_{29}N_2O_8FCl^+$, 587.1596. Found: 587.1603.

(Z)-3-benzyl-4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)imino)-6-(2-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)ethoxy)-3,4-dihydro-2H-benzo[e][1,3]oxazin-2-one (6b). The same method that was used in getting **6a** was adopted to prepare **6b**. (95% yield); mp 76–78°C; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 7.48-7.45 (q, J = 5.9 Hz, 1H, ArH), 7.42-7.40 (t, J = 5.9 Hz, 2H, ArH), 7.34-7.30 (overlap, 5H, ArH), 7.27-7.24 (overlap, 2H, ArH), 7.24-7.22 (m, 1H, ArH), 7.20-7.17 (m, 1H, ArH), 6.98-6.97 (d, J = 2.3 Hz, 1H, ArH), 6.78-6.76 (dd, J = 8.7, 2.3 Hz, 1H), 6.55 (s, 1H), 5.25 (s, 2H), 5.22 (s, 2H), 4.77 (br s, 3H), 4.58 (br s, 1H), 4.52 (s, 1H), 3.64 (s, 2H), 3.62-3.58 (m, 2H), 3.55 (s, 1H), 3.50-3.48 (m, 1H), 3.16-3.15 (m, 1H), 1.05-1.04 (d, J = 6.6 Hz, 3H); ^{13}C-NMR (DMSO-d_6, 600 MHz): δ 163.6, 162.0, 154.2, 150.0, 148.3, 145.8, 144.0, 143.1, 140.3, 137.3, 131.1, 128.9, 128.8, 128.0, 127.9, 127.6, 123.8, 123.4, 122.7, 120.9, 119.1 (d, J = 20 Hz), 116.5, 115.3, 115.1, 114.5 (d, J = 21 Hz), 111.7, 100.6, 72.4, 71.1, 70.9, 70.2, 69.0, 67.6, 65.0, 47.2, 18.3; ESI-MS 693.2 (M + H)+; HRMS (ESI): *Anal*. Calcd. for $C_{36}H_{35}N_2O_9FCl^+$, 693.2015. Found: 693.2043.

(Z)-4-((3-chloro-4-fluorophenyl)imino)-3-methyl-6-(2-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)ethoxy)-3,4-dihydro-2H-benzo[e][1,3]oxazin-2-one (6c). The same method that was used in getting **6a** was adopted to prepare **6c**. (91% yield); mp 75–77°C; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 7.46-7.43 (t, J = 9.2 Hz, 1H, ArH), 7.32-7.31 (d, J = 8.7 Hz, 1H, ArH), 7.23-7.21 (dd, J = 8.7, 2.8 Hz, 1H, ArH), 7.14-7.13 (d, J = 4.6 Hz, 1H, ArH), 6.92-6.91 (t, J = 3.9 Hz, 1H, ArH), 6.48 (br s, 1H, ArH), 4.76-4.75 (t, J = 3.7 Hz, 2H), 4.55-4.54 (t, J = 6.4 Hz, 2H), 3.70-3.64 (m, 3H), 3.56-3.50 (m, 3H), 3.45-3.42 (m, 1H), 3.34 (s, 3H), 3.19-3.16 (m, 1H), 1.09-1.08 (d, J = 5.9 Hz, 3H); ^{13}C-NMR (DMSO-d_6, 600 MHz): δ 154.7, 153.7 (d, J = 180 Hz), 148.3, 146.5, 145.7, 122.6, 122.5, 121.2, 120.2 (d, J = 36 Hz), 119.0, 118.5 (d, J = 54 Hz), 118.4, 118.3, 111.6, 100.6, 72.4,

71.1, 70.9, 69.0, 68.9, 67.7, 65.0, 18.4; ESI-MS 511.1 $(M+H)^+$; HRMS (ESI): *Anal*. Calcd. for $C_{23}H_{25}N_2O_8FCl^+$, 511.1283. Found: 511.1299.

(Z)-4-((3-chloro-4-((3-fluorobenzyl)oxy)phenyl)imino)-3-methyl-6-(2-(((2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)ethoxy)-3,4-dihydro-2H-benzo[e][1,3]oxazin-2-one (6d). The same method that was used in getting **6a** was adopted to prepare **6d**. (66% yield); mp 80–82°C; ^{1}H-NMR (DMSO-d_6, 600 MHz): δ 7.49-7.45 (q, J = 7.8 Hz, 1H, ArH), 7.33-7.26 (overlap, 3H, ArH), 7.26-7.25 (d, J = 8.7 Hz, 1H, ArH), 7.20-7.17 (overlap, 2H, ArH), 7.04-7.03 (d, J = 1.9 Hz, 1H, ArH), 6.85-6.84 (dd, J = 8.7, 2.3 Hz, 1H, ArH), 6.51 (br s, 1H), 5.22 (s, 2H), 4.76 (br s, 2H), 4.56 (br s, 1H), 4.52 (s, 1H), 3.63-3.61 (d, J = 13.7 Hz, 3H), 3.55 (s, 1H), 3.50-3.43 (m, 2H), 3.37 (s, 3H), 3.29-3.27 (m, 1H), 3.19-3.14 (m, 1H), 1.05-1.04 (d, J = 6.0 Hz, 3H); ^{13}C-NMR (DMSO-d_6, 600 MHz): δ 163.6, 162.0, 149.9, 145.6, 134.5, 140.2, 131.1, 131.0, 123.8, 123.7, 123.3, 122.4, 121.2, 119.2, 118.9 (d, J = 20 Hz), 116.4, 115.3, 115.2, 114.6, 114.5, 100.6, 72.4, 71.1, 70.9, 70.2, 69.0, 68.6, 67.6, 65.1, 18.3; ESI-MS 617.2 $(M+H)^+$; HRMS (ESI): *Anal*. Calcd. for $C_{30}H_{31}N_2O_9FCl^+$, 617.1702. Found: 617.1699.

Acknowledgments. This research was supported by the Natural Science Foundation of China (Grant No. 81373322). We are also grateful to Meiyu Geng from Shanghai Institute of Materia Medica, Chinese Academy of Sciences, for the biological assay.

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