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Research Article

Regioselective Synthesis of 3-(1*H*-indol-3-yl)-5-(1*H*-indole-3-carbonyl)-4-hydroxyfuroic Acids: Route to Hydroxyfuroic Acid-Based Insulin Receptor Activators

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ABSTRACT A new class of insulin receptor activator with a hydroxyfuroic acid in place of a hydroxyquinone moiety is reported. The synthesis of 3-(1*H*-indol-3-yl)-5-(1*H*-indole-3-carbonyl)-4-hydroxyfuroic acids (**26**–**30**) requires seven major steps. Key elements in the syntheses include (1) sequential preparation of two 4-(*N*-protected indole)-3-methoxy-furoic 2,5-dicarboxylic esters (**4** and **6**); (2) regioselective conversion of the furoic diacid **8** into its C-5 methyl ester 10 with methyl chloroformate; and (3) acylation of **10** by a 7-substituted indole under a mild condition. This study demonstrates a feasible route of synthesizing insulin receptor activators with a hydroxyfuroic acid scaffold. Among those hydroxyfuroic acid compounds, compound **28** demonstrates insulin receptor activation potential comparable to Merck's compound 2 with a dihydroxybenzoquinone scaffold. Drug Dev Res 72:247–258, 2011.

Key words: insulin receptor activator; regioselective synthesis; hydroxyfuroic acid

INTRODUCTION

Diabetes mellitus is a chronic disease characterized by poor glucose homeostasis resulting from either lack of insulin secretion by pancreatic islets or the irresponsiveness to insulin stimulation at the peripheral organs/tissues [Ross et al., 2004; Gale, 2001]. To bring the hyperglycemic conditions under control, several oral hypoglycemic agents are available on the market and can be categorized into five major classes based on their target receptors or enzymes. They include (1) sulfonylurea, an insulin secretagogue [Bryan et al., 2005]; (2) biguanide, an AMP kinase agonist [Zhou et al., 2001]; (3) α-glucosidase inhibitors [Hanefeld, 2007]; (4) peroxisome proliferator activated receptor (PPAR) agonists [Lee et al., 2003]; and (5) dipeptidyl peptidase IV inhibitors [Conarello et al., 2003; Tsai et al., 2008].

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Insulin receptor itself is an interesting target because an insulin receptor activator may potentially replace insulin at treating insulin-dependent diabetic patients, yet it is small enough to be orally active. Disclosed by Merck [Zhang et al., 1999], demethylasterriquinone B1 (DAQ B1), extracted from tropical fungus, *Pseudomassaria* sp., and its structurally simplified derivative "Compound 2," are capable of activating insulin receptor in the absence of insulin [Liu et al., 2000; Strowski et al., 2004] (Fig. 1). Interestingly, bioconverted products of DAQ B1, 4-hydroxyfuroic acids, have been demonstrated to be insulin receptor activators and display a hypoglycemic effect on *db/db* mice [Chen et al., 2003]. Additionally, kojic acid compounds, also derivatives of DAQ B1, have been shown to be capable of promoting insulin receptor phosphorylation [Xiong and Pirrung, 2008; Pirrung et al., 2008]. Our recent report has presented an approach for preparing simplified analogues of 4-hydroxyfuroic acids, designated series 1 compounds, in which the C-3 reverse prentyl- and C-5 prentyl indole rings were replaced with a phenyl and a 7-substituted indole, respectively [Chou et al., 2006]. However, series 1 compounds showed no appreciable potential at activating insulin receptor. Therefore, we describe the synthesis of structurally less modified analogues, designated series 2 compounds, in which the C-3 and C-5 indole rings of DAQ B1 are replaced by an unsubstituted C-3 indole and a C-5 (hydroxyalkyl)-indole or C-5 (carboxyalkyl)indole (Fig. 1).

EXPERIMENTAL

General Remarks

Melting points were measured in open capillary tubes using an Büchi immersion apparatus, and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker-AC 500 (500 MHz) spectrometer (chemical shifts in ppm). Mass spectra (electron spray-ES) were recorded on a JEOL-JMSD-D-100 instrument. Exact mass spectra were measured by a JEOL-JMSD-HX100 high-resolution mass spectrometer.

3-Hydroxy-4-[1-(4-methoxy-benzyl)-1*H*-indol-3-yl]-furan-2,5-dicarboxylic acid dimethyl ester (3)

A suspension of potassium *t*-butoxide was prepared by reacting potassium (37.7 g, 0.96 mol, ~300 mol% vs **1**) and *t*-butanol (94 ml, 0.99 mol) in dry benzene (1,200 ml). The suspension was then kept under reflux. A solution of dimethyl diglycolic acid ester **1** (52.1 g, 0.32 mol) and [1-(4-methoxy-benzyl)-1*H*- indol-3-yl]-oxo-acetic acid methyl ester (**2**) (136.6 g, 0.42 mol, ~130 mol% vs **1**) in benzene (1,000 ml) was added dropwise to the above refluxing suspension. After being refluxed under nitrogen for 2.5 h, the mixture was cooled, diluted with ice water, and acidified with 3 N HCl. The resultant mixture was diluted with ethyl acetate (500 ml); the separated organic layer was washed with water, dried, and concentrated to yield **3** (194.0 g) as a crude product. An analytical sample was prepared by chromatographic

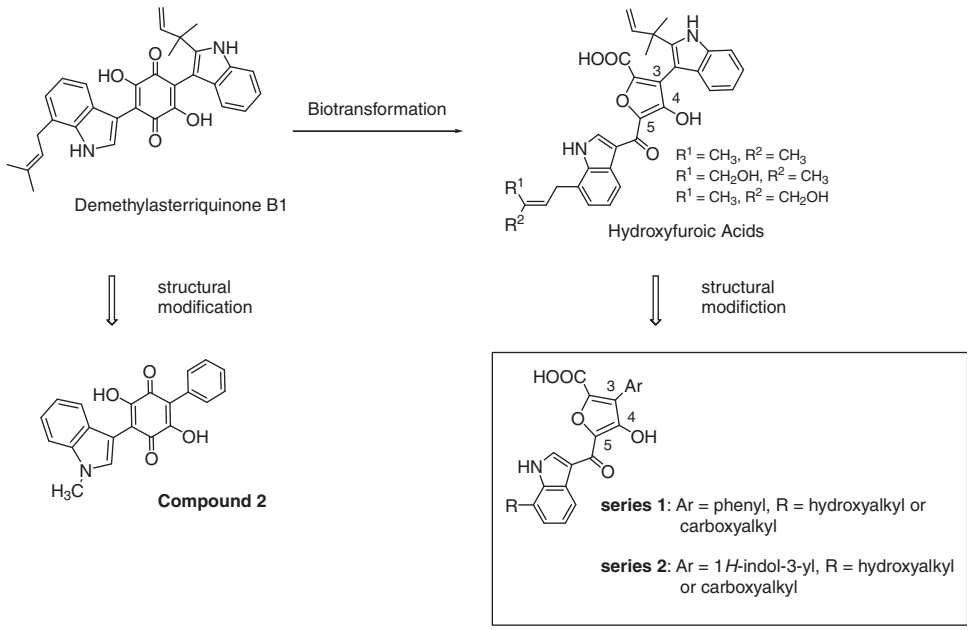


Fig. 1. Rational design of 3-phenyl- or 3-(1*H*-indol-3-yl)-4-hydroxy furoic acid derivatives as potential insulin receptor activators (series 1 and series 2, respectively).

purification using 1/2 ethyl acetate-hexane as an eluent to give **3a** as yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.73 (s, 6H), 3.97 (s, 3H), 5.26 (s, 2H), 6.81 (d, J = 8.6 Hz, 2H), 7.11 (d, J = 8.6 Hz, 2H), 7.13–7.15 (m, 1H), 7.32 (d, J = 7.9 Hz, 1H), 7.51 (s, 1H), 7.62 (d, J = 7.9 Hz, 1H). MS (ESI): 436.0 (M^++H); HREIMS: Calculated for $C_{24}H_{21}NO_7$ (M^+) 435.1318, found 435.1321.

3-Methoxy-4-[1-(4-methoxy-benzyl)-1*H*-indol-3-yl]-furan-2,5-dicarboxylic acid dimethyl ester (4)

Potassium carbonate (238.8 g, 1.73 mol) and MeI (183.2 g, 0.572 mol) were added sequentially to a solution of the crude **3** (194.0 g, obtained from the previous step) in DMF (3,750 ml). After being stirred at room temperature for 24 h, the reaction mixture was filtered from a sintered glass funnel. The filtrate was concentrated under vacuum at <50°C. The residue thus obtained was diluted with dichloromethane (1,000 ml) and washed with brine and water. The separated organic layer was concentrated, and the residue was purified by silica gel column chromatography using 1/4 ethyl acetate-hexane as an eluent to produce **4** (93.0 g, yield in two steps: 64.4%) as a white powder, mp: 116–118°C (1/4 ethyl acetate-hexane). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.78 (s, 3H), 3.79 (s, 3H), 3.80 (s, 3H), 3.97 (s, 3H), 5.32 (s, 1H), 6.85 (d, J = 8.6 Hz, 1H), 7.15 (t, J = 8.6 Hz, 3H), 7.22 (t, J = 7.6 Hz, 1H), 7.34 (d, J = 8.1 Hz, 1H), 7.48 (s, 1H), 7.54 (d, J = 7.9 Hz, 1H). MS (ESI): 450.0 (M^++H); HREIMS: Calculated for $C_{25}H_{23}NO_7$ (M^+) 449.1475, found 449.1477.

3-(1*H*-indol-3-yl)-4-methoxy-furan-2,5-dicarboxylic acid dimethyl ester (5)

DDQ (25.9 g, 0.11 mol) was added to a solution of **4** (25.6 g, 0.057 mol) in CH_2Cl_2 (770 ml) and H_2O (43 ml). After the reaction mixture was stirred at room temperature for 21 h, it was placed on an ice-water bath; 5% Na_2CO_3 (500 ml) was then added with stirring. The resultant mixture was diluted with CH_2Cl_2 (300 ml) and washed with brine and water. The separated organic layer was dried and concentrated. The crude product was purified by silica gel column chromatography using 1/2 ethyl acetate-hexane as an eluent to afford **5** (11.3 g, 60.2%) as a white powder, mp: 150–151°C (1/2 ethyl acetate-hexane). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 3.77 (t, J = 2.9 Hz, 3H), 3.82 (s, 3H), 3.97 (d, J = 4.3 Hz, 3H), 7.16 (t, J = 7.6 Hz, 1H), 7.24 (t, J = 7.6 Hz, 1H), 7.41 (d, J = 8.1 Hz, 1H), 7.53–7.55 (m, 2H), 8.52 (s, 1H). MS (ESI): 330.3 (M^++H); HREIMS: Calculated for $C_{17}H_{15}NO_6$ (M^+) 329.0899, found 329.0897.

3-Methoxy-4[1-(toluene-4-sulfonyl)-1*H*-indol-3-yl]-furan-2,5-dicarboxylic acid dimethyl ester (6)

Method A. Intermediate **5** (15.1 g, 0.046 mol) was mixed with TsCl (17.5 g, 0.092 mol) and K_2CO_3 (19.0 g, 0.14 mol) in 2-butanone (460 ml). After the mixture was refluxed under nitrogen for 2 h, additional amounts of TsCl (8.7 g, 0.046 mol) and K_2CO_3 (9.5 g, 0.069 mol) were added. The resultant mixture was then refluxed overnight. The reaction mixture was filtered from a sintered glass funnel. The filtrate was concentrated under vacuum at <40°C, and the residue was treated with ice-cooled *t*-BuOMe (100 ml) to form a suspension. The suspension was again filtered from a sintered glass funnel. The precipitate was collected and dried to afford **6** (20.4 g, 91.9%) as a white powder, mp 107–108°C (1/2 (v/v) ethyl acetate-hexane). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.37 (s, 3H), 3.78 (s, 3H), 3.81 (s, 3H), 4.00 (s, 3H), 7.27 (d, J = 8.2 Hz, 2H), 7.28–7.29 (m, 1H), 7.34–7.36 (m, 1H), 7.40–7.42 (m, 1H), 7.87 (d, J = 8.2 Hz, 2H), 7.89 (s, 1H), 8.03 (d, J = 8.3 Hz, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 22.0, 52.7 (overlapping peaks), 62.6, 110.1; 114.0, 120.8, 121.4, 123.9, 125.3, 127.4, 127.7, 129.7, 130.4, 133.4, 135.0, 135.4, 140.7, 145.6, 153.6, 158.7 (overlapping peaks). MS (ESI): 484.0 (M^++H); HREIMS: Calculated for $C_{24}H_{21}NO_8S$ (M^+) 483.0988, found 483.0975. Anal. Calcd for $C_{24}H_{21}NO_8S$: C, 59.62; H, 4.38; N, 2.90. Found: C, 59.77; H, 4.36; N, 2.85.

Method B. A solution of *t*-butyllithium in pentane (24.8 ml of 1.7 M solution; 42.0 mmol) was added to a stirred solution of 1-tosyl-3-iodoindole (7.94 g, 20.0 mmol) in anhydrous THF (100 ml) at −78°C in a nitrogen atmosphere. The solution was stirred at this temperature for 0.5 h; trimethyl borate (22.8 ml, 20.9 g, 200.0 mmol) was dissolved in THF (400 ml) and pre-cooled to −78°C and then added via a cannula. The reaction mixture was stirred at −78°C for 1 h; 8 ml of 50% MeOH was then added, and the solution was left to reach room temperature. The reaction mixture was poured into water, phases were separated, and the aqueous phase was extracted with ether. The organic phases were combined, dried ($MgSO_4$), and evaporated. The crude oily boronic acid was triturated with hexane-acetone to yield the boronic acid **8** as an amorphous white powder (2.8 g, 44.4%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 2.30 (s, 3H), 7.06 (d, J = 7.7 Hz, 1H), 7.41–7.47 (m, 2H), 7.79 (d, J = 7.7 Hz, 2H), 7.97 (d, J = 7.5 Hz, 1H), 8.04 (d, J = 7.5 Hz, 1H), 8.35 (d, J = 7.0 Hz, 1H), 8.62 (s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 145.7, 138.7, 136.2, 135.2, 135.1, 133.8, 130.5, 127.4, 125.2, 124.5, 123.5, 113.9, 21.9. MS (ESI): 316 (M^++H); HREIMS: Calculated for $C_{15}H_{14}BNO_4S$ (M^+) 315.0737, found 315.0748. Anal.

Calcd for $C_{15}H_{14}BNO_4S$: C, 57.17; H, 4.48; N, 4.44. Found: C, 57.20; H, 4.43; N, 4.35. A mixture of the triflate **7** (0.36 g, 1.0 mmol), bis-(triphenylphosphine) palladium (II) chloride (0.07 g, 0.1 mmol), the boric acid **8** (0.38 g, 1.2 mmol), Na_2CO_3 (0.32 g, 3.0 mmol) and DMF (10 ml) was stirred at 90°C for 12 h. The solvent was removed in vacuo, and the residue was diluted with ethyl acetate and water. The separated organic layer was washed with water, dried ($MgSO_4$), and evaporated. The residue was subjected to flash chromatography on silica gel using 1/2 (v/v) ethyl acetate-hexane to afford **6** (0.32 g, 65.5%) as a white powder. The product is identical to that prepared by condensation (Method A).

3-Methoxy-4-[1-(toluene-4-sulfonyl)-1*H*-indol-3-yl]-furan-2,5-dicarboxylic acid (9)

Lithium hydroxide (4.3 g, 177.7 mmol, 700 mol% vs intermediate **6**) was added at 25°C to a solution of intermediate **6** (12.3 g, 25.4 mmol) in methanol (280 ml) and H_2O (23 ml). The resultant solution was stirred at 25°C for 5 h, acidified with 10% sulfuric acid, and concentrated. The residue was diluted with ethyl acetate and was washed with water and brine, dried, and concentrated to give **9** (11.5 g, 99.3%) as a white powder, mp 153–155°C (ethyl acetate). ^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 2.35 (s, 3H), 3.84 (s, 3H), 7.26–7.30 (m, 1H), 7.37–7.50 (m, 3H), 7.50 (d, J = 7.9 Hz, 1H), 7.93 (d, J = 8.3 Hz, 1H), 8.00 (s, 1H), 8.05 (d, J = 8.3 Hz, 1H). ^{13}C-NMR (125 MHz, ^{6}d-acetone) δ: 158.7, 158.4, 153.7, 146.1, 141.2, 135.3, 135.0, 133.8, 130.5, 130.4, 127.8, 127.4, 125.2, 123.8, 121.6, 120.4, 113.8, 111.1, 62.1, 21.0. MS (ESI): 456.0 (M^++H); HREIMS: Calculated for $C_{22}H_{17}NO_8S$ (M^+) 455.0675, found 455.0672. Anal. Calcd for $C_{22}H_{17}NO_8S$: C, 58.02; H, 3.76; N, 3.08. Found: C, 58.05; H, 3.73; N, 3.11.

3-Methoxy-4-[1-(toluene-4-sulfonyl)-1*H*-indol-3-yl]-furan-2,5-dicarboxylic acid 5-methyl ester (10)

The typical procedure for the regioselective synthesis of the half ester **10** involved the following steps. To a solution of the diacid **9** (8.0 g, 17.6 mmol) and triethylamine (1.9 g, 18.8 mmol, 107 mole% vs **9**) in dichloromethane (106 ml) at 0°C was added a solution of methyl chloroformate (1.70 g, 18.0 mmol, 103 mole% vs **9**) in dichloromethane (2 ml). After stirring at 0°C for 60 min, DMAP (0.26 g) was added. The resulting solution was stirred at 0°C for 1 h and at room temperature for a further 18 h, acidified with 10% sulfuric acid, and concentrated. The residue was diluted with ethyl acetate, washed with water and brine, dried, and concentrated. Half esters **10** and **10′** were isolated from the mixture by silica gel column chromatography using 1/2 (v/v) ethyl acetate/hexane through ethyl acetate to 1/20 (v/v) methanol/ethyl acetate as an eluent to yield successively the diester **6** (1.3 g, 15.3%), **10′** (0.64 g, 7.8%), **10** (4.5 g, 54.6%), and recovered **9** (1.7 g, 21.2%). **10′**, a white powder, mp 148–150°C (methanol). ^{1}H-NMR (500 MHz, ^{6}d-DMSO) δ: 2.30 (s, 3H), 3.66 (s, 3H), 3.83 (s, 3H), 7.21–7.22 (m, J = 7.5, 1H), 7.30–7.32 (m, 1H), 7.35 (s, 1H), 7.37 (d, J = 8.1 Hz, 2H), 7.88–7.91 (m, 4H). ^{13}C-NMR (125 MHz, ^{6}d-DMSO) δ: 21.9, 52.3, 62.5, 113.1, 113.5, 113.8, 122.2, 124.1, 125.3, 127.4, 127.7, 129.9, 131.0, 131.1, 134.7, 134.9, 146.3, 150.8, 154.5, 159.1, 162.4. MS (ESI): 470.0 (M^++H); HREIMS: Calculated for $C_{24}H_{21}NO_8S$ (M^+) 469.4639, found 469.4645. Anal. Calcd for $C_{23}H_{19}NO_8S$: C, 58.84; H, 4.08; N, 2.98. Found: C, 58.87; H, 4.33; N, 2.71. **10**, a white powder, mp 139–140°C (methanol). ^{1}H-NMR (500 MHz, ^{6}d-DMSO) δ: 2.33 (s, 3H), 3.68 (s, 3H), 3.74 (s, 3H), 7.26–7.27 (m, 1H), 7.29–7.44 (m, 4H), 7.88–7.92 (m, 2H), 7.97–8.02 (m, 2H). ^{13}C-NMR (125 MHz, ^{6}d-DMSO) δ: 21.9, 53.0, 62.9, 110.9, 114.0, 120.6, 121.9, 124.5, 125.8, 127.7, 128.0, 130.1, 131.2, 134.6, 134.7, 134.8, 140.4, 146.6, 153.1, 158.7, 159.4. MS (ESI): 470.0 (M^++H); HREIMS: Calculated for $C_{24}H_{21}NO_8S$ (M^+) 469.4639, found 469.4643. Anal. Calcd for $C_{23}H_{19}NO_8S$: C, 58.84; H, 4.08; N, 2.98. Found: C, 58.75; H, 4.37; N, 2.75.

General procedure for the synthesis of 4-methoxyfuroic acid esters 16–20

Oxalyl chloride (10 ml/g **10**) was added to **10** (12.2 mmol) and stirred for 20 min. The mixture was condensed under vacuum to give the acyl chloride of **10**. In a separated flask, a 60-ml dichloromethane solution containing 12.4 mmol of the 7-substituted indoles **11**–**15** was added with Et_2AlCl (1 M in hexane, 30 mmol) at 0°C and stirred for 0.5 h before 50 ml dichloromethane solution containing the aforementioned acyl chloride of **10** was added. After 1 h stirring at 0°C and 14 h at room temperature, the reaction was quenched with 3 N HCl. The separated organic layer was condensed and the residue was purified by silica gel chromatography to yield **16**–**20** (51–66% yield).

5-[7-(3-Acetoxy-propyl)-1*H*-indole-3-carbonyl]-4-methoxy-3[1-(toluene-4-sulfonyl)-1*H*-indol-3-yl]-furan-2-carboxylic acid methyl ester (16)

^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 2.12–2.14 (m, 2H), 2.36 (s, 3H), 3.06 (t, J = 7.5 Hz, 2H), 3.76 (s, 3H), 3.91 (s, 3H), 4.12 (t, J = 6.6 Hz, 2H), 7.17–7.18 (m, 1H), 7.22–7.25 (m, 1H), 7.29–7.30 (m, 1H), 7.39–7.40 (m, 1H), 7.42 (d, J = 8.3 Hz, 2H), 7.51 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.3 Hz, 2H), 7.99 (s, 1H), 8.06 (d, J = 8.4 Hz, 1H), 8.39 (d, J = 8.0 Hz, 1H), 8.66

(d, J = 3.2 Hz, 1H). MS (ESI): 669.0 (M^++H); HREIMS: Calculated for $C_{36}H_{32}N_2O_9S$ (M^+) 668.1828, found 668.1831.

5-[7-(3-Acetoxy-butyl)-1*H*-indole-3-carbonyl]-4-methoxy-3[1-(toluene-4-sulfonyl)-1*H*-indol-3-yl]-furan-2-carboxylic acid methyl ester (17)

^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 1.75–1.77 (m, 2H), 1.84–1.86 (m, 2H), 1.99 (s, 3H), 2.38 (s, 3H), 3.04 (t, J = 7.5 Hz, 2H), 3.79 (s, 3H), 3.94 (s, 3H), 4.10 (t, J = 6.6 Hz, 2H), 7.18–7.19 (m, 1H), 7.22–7.25 (m, 1H), 7.31–7.32 (m, 1H), 7.39–7.40 (m, 1H), 7.43 (d, J = 8.4 Hz, 2H), 7.53 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 8.4 Hz, 2H), 8.01 (s, 1H), 8.08 (d, J = 8.4 Hz, 1H), 8.40 (d, J = 7.8 Hz, 1H), 8.67 (d, J = 3.2 Hz, 1H). MS (ESI): 683.0 (M^++H); HREIMS: Calculated for $C_{37}H_{34}N_2O_9S$ (M^+) 682.1985, found 682.1983.

5-[7-(3-Acetoxy-pentyl)-1*H*-indole-3-carbonyl]-4-methoxy-3[1-(toluene-4-sulfonyl)-1*H*-indol-3-yl]-furan-2-carboxylic acid methyl ester (18)

^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 1.48–1.50 (m, 2H), 1.67–1.69 (m, 2H), 1.75–1.85 (m, 2H), 1.98 (s, 3H), 2.37 (s, 3H), 3.00 (t, J = 7.5 Hz, 2H), 3.77 (s, 3H), 3.92 (s, 3H), 4.05 (t, J = 6.9 Hz, 2H), 7.16–7.17 (m, 1H), 7.22–7.25 (m, 1H), 7.30–7.31 (m, 1H), 7.39–7.39 (m, 1H), 7.40 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 7.9 Hz, 1H), 7.97 (d, J = 8.4 Hz, 2H), 8.00 (s, 1H), 8.07 (d, J = 8.4 Hz, 1H), 8.40 (d, J = 7.8 Hz, 1H), 8.67 (d, J = 3.2 Hz, 1H). MS (ESI): 697.0 (M^++H); HREIMS: Calculated for $C_{38}H_{36}N_2O_9S$ (M^+) 696.2142, found 696.2138.

4-Methoxy-5-[7-(4-methoxycarbonyl-butyl)-1*H*-indole-3-carbonyl]-3-[1-(toluene-4-sulfonyl)-1*H*-indol-3-yl]-furan-2-carboxylic acid methyl ester (19)

^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 1.63–1.80 (m, 4H), 2.34–2.36 (m, 2H), 2.93 (s, 3H), 3.60 (t, J = 5.8 Hz, 2H), 3.76 (s, 3H), 3.88 (s, 3H), 3.94 (s, 3H), 7.20–7.30 (m, 2H), 7.3–7.40 (m, 4H), 7.47 (d, J = 7.9 Hz, 1H), 7.90–7.95 (m, 3H), 8.00 (s, 1H), 8.05–8.10 (m, 1H), 8.40 (d, J = 3.2 Hz, 1H). MS (ESI): 683.0 (M^++H); HREIMS: Calculated for $C_{37}H_{34}N_2O_9S$ (M^+) 682.1985, found 682.1983.

5-[7-(5-Acetoxy-4-acetoxymethyl-pentyl)-1*H*-indol-3-carbonyl]-4-methoxy-3-[(1-(toluene-4-sulfonyl)-1*H*-indol-3-yl)-furan-2-carboxylic acid methyl ester (20)

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.25–1.27 (m, 1H), 1.46–1.48 (m, 2H), 1.82–1.84 (m, 2H), 2.10 (s, 6H), 2.35 (s, 3H), 2.87 (t, J = 8.1 Hz, 2H), 3.76 (s, 3H), 3.83 (s, 3H), 4.12–4.14 (m, 4H), 7.12 (d, J = 7.2 Hz, 1H), 7.24–7.28 (m, 4H), 7.34–7.36 (m, 1H), 7.46 (d, J = 7.9 Hz, 1H), 7.83–7.85 (m, 3H), 8.03 (d, J = 8.3 Hz, 1H), 8.46 (d, J = 8.0 Hz, 1H), 8.60 (d, J = 3.0 Hz, 1H), 9.74 (br s, 1H). MS (ESI): 769.5 (M^++H); HREIMS: Calculated for $C_{41}H_{40}N_2O_{11}S$ (M^+) 768.2353, found 768.3348.

General procedure for the synthesis of 4-hydroxyfuroic acid esters 21–25

A 50-ml dichloromethane solution containing 5.3 mmol of **16–20** was added with BCl_3 (1 M in hexane, 35 mmol) at 0°C. The reaction mixture was stirred at 0°C for 0.5 h and at room temperature for 3 h, and then diluted with additional 50 ml dichloromethane. After another 0.5 h of stirring, the mixture was quenched with ice water and the separated organic layer was condensed. The residue was purified by silica gel chromatography to yield **21–25** (80–85% yield).

5-[7-(3-Acetoxy-propyl)-1*H*-indole-3-carbonyl]-4-hydroxy-3[1-(toluene-4-sulfonyl)-1*H*-indol-3-yl]-furan-2-carboxylic acid methyl ester (21)

^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 2.10–2.12 (m, 2H), 2.39 (s, 3H), 3.10 (t, J = 7.9 Hz, 2H), 3.86 (s, 3H), 4.15 (t, J = 6.6 Hz, 2H), 7.23 (d, J = 7.2 Hz, 1H), 7.28–7.30 (m, 1H), 7.31–7.33 (m, 1H), 7.42–7.44 (m, 1H), 7.45 (d, J = 8.3 Hz, 2H), 7.65 (d, J = 7.9 Hz, 1H), 8.00 (d, J = 8.3 Hz, 2H), 8.08–8.10 (m, 1H), 8.11 (s, 1H), 8.37 (d, J = 8.0 Hz, 1H), 8.80 (d, J = 3.2 Hz, 1H). MS (ESI): 655.0 (M^++H); HREIMS: Calculated for $C_{35}H_{30}N_2O_9S$ (M^+) 654.1672, found 654.1668.

5-[7-(3-Acetoxy-butyl)-1*H*-indole-3-carbonyl]-4-hydroxy-3[1-(toluene-4-sulfonyl)-1*H*-indol-3-yl]-furan-2-carboxylic acid methyl ester (22)

^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 1.77–1.79 (m, 2H), 1.85–1.87 (m, 2H), 2.00 (s, 3H), 2.39 (s, 3H), 3.06 (t, J = 7.8 Hz, 2H), 3.87 (s, 3H), 4.11 (t, J = 6.6 Hz, 2H), 7.21–7.23 (m, 1H), 7.31–7.32 (m, 2H), 7.41–7.43 (m, 1H), 7.44 (d, J = 8.4 Hz, 2H), 7.65 (d, J = 7.9 Hz, 1H), 8.00 (d, J = 8.4 Hz, 2H), 8.10 (d, J = 8.4 Hz, 1H), 8.11 (s, 1H), 8.37 (d, J = 7.8 Hz, 1H), 8.79 (s, J = 3.3 Hz, 1H). MS (ESI): 669.0 (M^++H); HREIMS: Calculated for $C_{36}H_{32}N_2O_9S$ (M^+) 668.1829, found 668.1833.

5-[7-(3-Acetoxy-pentyl)-1*H*-indole-3-carbonyl]-4-hydoxy-3[1-(toluene-4-sulfonyl)-1*H*-indol-3-yl]-furan-2-carboxylic acid methyl ester (23)

^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 1.48–1.50 (m, 2H), 1.67–1.69 (m, 2H), 1.75–1.85 (m, 2H), 1.97 (s, 3H), 2.37 (s, 3H), 3.00 (t, J = 7.5 Hz, 2H), 3.84 (s, 3H), 4.04 (t, J = 6.9 Hz, 2H), 7.15–7.17 (m, 1H), 7.18–7.20 (m, 1H), 7.25–7.30 (m, 2H), 7.30–7.40 (m, 3H), 7.65 (d, J = 7.9 Hz, 1H), 7.98 (d, J = 8.4 Hz, 2H), 8.07

(d, J = 8.4 Hz, 1H), 8.10 (s, 1H), 8.30 (d, J = 7.8 Hz, 1H), 8.70 (s, 1H). MS (ESI): 683.0 (M^++H); HREIMS: Calculated for $C_{37}H_{34}N_2O_9S$ (M^+) 682.1985, found 682.1983.

4-Hydroxy-5-[7-(4-methoxycarbonyl-butyl)-1*H*-indole-3-carbonyl]-3-[1-(toluene-4-sulfonyl)- 1*H*-indol-3-yl]-furan-2-carboxylic acid methyl ester (24)

^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 1.63–1.80 (m, 4H), 2.34–2.36 (m, 2H), 2.97–2.99 (m, 3H), 3.59 (s, 3H), 3.61 (s, 3H), 3.78 (t, J = 5.8 Hz, 2H), 7.16 (d, J = 7.3 Hz, 2H), 7.20–7.30 (m, 2H), 7.30–7.40 (m, 4H), 7.80–7.95 (m, 2H), 8.00 (s, 1H), 8.10–8.15 (m, 1H), 8.27 (s, 1H), 8.28 (d, J = 7.4 Hz, 1H). MS (ESI): 669.0 (M^++H); HREIMS: Calculated for $C_{36}H_{32}N_2O_9S$ (M^+) 668.1829, found 668.1833.

5-[7-(5-Acetoxy-4-acetoxymethyl-pentyl)-1*H*-indol-3-carbonyl]-4-hydroxy-3-[(1-(toluene-4-sulfonyl)-1*H*-indol-3-yl)-furan-2-carboxylic acid methyl ester (25)

^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.25–1.27 (m, 1H), 1.47–1.49 (m, 2H), 1.83–1.85 (m, 2H), 2.12 (s, 6H), 2.35 (s, 3H), 2.87 (t, J = 8.2 Hz, 2H), 3.81 (s, 3H), 4.10–4.12 (m, 2H), 4.18–4.20 (m, 2H), 7.14 (d, J = 7.2 Hz, 1H), 7.25–7.28 (m, 4H), 7.34–7.36 (m, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.85 (d, J = 8.2 Hz, 2H), 7.94 (s, 1H), 8.02 (d, J = 8.4 Hz, 1H), 8.39 (d, J = 7.9 Hz, 1H), 8.73 (s, 1H), 9.96 (br s, 1H). MS (ESI): 755.5 (M^++H); HREIMS: Calculated for $C_{40}H_{38}N_2O_{11}S$ (M^+) 764.2196, found 754.2193.

General procedure for the synthesis of 4-hydroxyfuroic acid derivatives 26–30

A methanol solution (10–15 ml/g) containing ~0.11 g of the furan bisindole (**21**–**25**) from the previous step was added with 1.0 ml of 5% aqueous NaOH and refluxed for 0.5 h. The mixture was concentrated, acidified with 3 N HCl, and then extracted with ethyl acetate. The extract was washed with brine, dried, and solvent evaporated. The residue was purified by silica gel chromatography to give the title compounds with 66–70% yield.

4-Hydroxy-5[7-(3-hydroxy-propyl)-1*H*-indole-3-carbonyl]-3-(1*H*-indol-3-yl)-furan-2-carboxylic acid (26)

A yellow powder, mp 140–142°C (ethyl acetate). ^{1}H-NMR (500 MHz, ^{6}d-DMSO) δ: 1.84–1.88 (m, 2H), 2.95 (t, J = 7.4 Hz, 2H), 3.51 (t, J = 6.5 Hz, 2H), 7.02–7.05 (m, 1H), 7.11–7.15 (m, 2H), 7.18–7.21 (m, 1H), 7.46 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.62–7.63 (m, 1H), 8.27 (d, J = 7.5 Hz, 1H), 8.66 (d, J = 2.2 Hz, 1H). ^{13}C-NMR (125 MHz, ^{6}d-DMSO) δ: 27.8, 35.2, 61.1, 103.4, 112.4, 114.3, 116.9, 119.7, 120.2, 121.5, 121.7, 121.9, 123.2, 123.7, 126.8, 127.2, 127.7, 135.2, 136.0, 136.3, 136.4, 136.7, 158.7, 161.6, 178.3. MS (ESI, negative mode): 443.2 (M^+–H); HREIMS: Calculated for $C_{25}H_{20}N_2O_6$ (M^+) 444.1321, found 444.1324. Anal. Calcd for $C_{25}H_{20}N_2O_6$: C, 67.56; H, 4.54; N, 6.30. Found: C, 67.61; H, 4.52; N, 5.98.

4-Hydroxy-5-[7-(3-hydroxy-butyl)-1*H*-indole-3-carbonyl]-3-(1*H*-indol-3-yl)-furan-2-carboxylic acid (27)

A yellow powder, mp 145–147°C (ethyl acetate). ^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 1.63–1.65 (m, 2H), 1.84–1.86 (m, 2H), 3.01 (t, J = 7.5 Hz, 2H), 3.64 (t, J = 6.5 Hz, 2H), 7.07–7.08 (m, 1H), 7.15–7.17 (m, 2H), 7.23–7.25 (m, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.35–7.36 (m, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.79–80 (m, 1H), 8.35 (d, J = 8.0 Hz, 1H), 8.60 (br s, 1H), 8.90 (br s, 1H). ^{13}C-NMR (125 MHz, ^{6}d-DMSO) δ: 27.2, 30.7, 33.1, 61.5, 102.8, 112.5, 114.3, 119.9, 120.0, 120.2, 121.4, 122.0, 123.4, 123.9, 127.0, 127.5, 127.9, 135.4, 136.0, 136.5, 136.8, 139.6, 143.7, 153.3, 160.7, 178.5. MS (ESI, negative mode): 457.2 (M^+-H); HREIMS: Calculated for $C_{26}H_{22}N_2O_6$ (M^+) 458.1478, found 458.1459. Anal. Calcd for $C_{26}H_{22}N_2O_6$: C, 68.11; H, 4.84; N, 6.11. Found: C, 68.23; H, 4.78; N, 5.88.

4-Hydroxy-5[7-(3-hydroxy-pentyl)-1*H*-indole-3-carbonyl]-3-(1*H*-indol-3-yl)-furan-2-carboxylic acid (28)

A yellow powder, mp 158–160°C (ethyl acetate). ^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 1.53–1.55 (m, 2H), 1.61 (t, J = 7.2 Hz, 2H), 1.82 (t, J = 7.4 Hz, 2H), 3.01 (t, J = 7.8 Hz, 2H), 3.58 (t, J = 6.2 Hz, 2H), 7.10–7.11 (m, 1H), 7.18–7.20 (m, 2H), 7.26–7.28 (m, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 2.5 Hz, 1H), 8.38 (d, J = 7.9 Hz, 1H), 8.84 (d, J = 3.2 Hz, 1H), 10.7 (br s, 1H), 11.4 (br s, 1H). ^{13}C-NMR (125 MHz, ^{6}d-acetone) δ: 26.2, 30.5, 31.4, 33.0, 62.0, 102.9, 111.9, 114.2, 119.7, 120.0, 120.3, 121.4, 121.9, 123.2, 123.9, 126.8, 127.1, 127.3, 127.5, 135.2, 135.9, 136.2, 136.8, 139.1, 155.2, 159.7, 179.6. MS (ESI): 473.0 (M^++H); HREIMS: Calculated for $C_{27}H_{24}N_2O_6$ (M^+) 472.1634, found 472.1639. Anal. Calcd for $C_{27}H_{24}N_2O_6$: C, 68.63; H, 5.12; N, 5.93. Found: C, 68.47; H, 4.98; N, 5.87.

5-[7-(4-Carboxy-butyl)-1*H*-indole-3-carbonyl]-4-hydroxy-3-(1*H*-indol-3-yl)-furan-2-carboxylic acid (29)

An amorphous yellow powder. ^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 1.74 (d, J = 7.6 Hz, 2H), 1.92 (d, J = 2.1 Hz, 2H), 2.38 (t, J = 7.5 Hz, 2H), 3.02 (t, J = 7.5 Hz, 2H), 7.08–7.11 (m, 1H), 7.17–7.21

(m, 2H), 7.25–7.27 (m, 1H), 7.50 (d, J = 8.1 Hz, 1H), 7.67 (d, J = 7.8 Hz, 1H), 7.77 (d, J = 2.4 Hz, 1H), 8.37 (d, J = 7.8 Hz, 1H), 8.83 (d, J = 3.0 Hz, 1H), 10.66 (br s, 1H),11.44 (br s, 1H). ^{13}C-NMR (125 MHz, ^{6}d-acetone) δ: 25.1, 30.0, 31.0, 33.5, 102.9, 111.9, 114.3, 119.7, 120.0, 120.4, 121.4, 121.9, 123.3, 123.9, 126.7, 127.0, 127.3, 127.5, 135.2, 135.8, 136.2, 136.8, 139.1, 155.1, 159.6, 174.3, 179.6. MS (ESI): 486.5 (M^+); HREIMS: Calculated for $C_{27}H_{22}N_2O_7$ (M^+) 482.1427, found 482.1432. Anal. Calcd for $C_{27}H_{22}N_2O_7$: C, 66.66; H, 4.56; N, 5.76. Found: C, 66.57; H, 4.53; N, 5.68.

4-Hydroxy-5-[7-(5-hydroxy-4-hydroxymethyl-pentyl)-1*H*-indole-3-carbonyl]-3(1*H*-indol-3-yl)-furan-2-carboxylic acid (30)

A yellow powder, mp 141–143°C (ethyl acetate). ^{1}H-NMR (500 MHz, ^{6}d-acetone) δ: 1.50–1.53 (m, 2H), 1.74–1.76 (m, 1H), 1.85–1.89 (m, 2H), 3.01 (t, J = 7.9 Hz, 2H), 3.63–3.67 (m, 4H), 7.09–7.12 (m, 1H), 7.17–7.21 (m, 2H), 7.25–7.28 (m, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.79 (d, J = 2.6 Hz, 1H), 8.38 (d, J = 7.8 Hz, 1H), 8.84 (d, J = 3.1 Hz, 1H), 10.68 (br s, 1H), 11.52 (br s, 1H). ^{13}C-NMR (125 MHz, ^{6}d-acetone) δ: 28.4, 28.5, 31.7, 43.4, 64.0, 103.0, 111.8, 114.2, 119.7, 120.2, 121.4, 121.9, 123.2, 123.9, 124.1, 126.9, 127.1, 127.3, 127.5, 135.1, 135.3, 135.9, 136.1, 136.8, 137.8, 155.2, 179.6. MS (ESI): 503.0 (M^++H); HREIMS: Calculated for $C_{28}H_{26}N_2O_7$ (M^+) 502.1740, found 502.1746. Anal. Calcd for $C_{28}H_{26}N_2O_7$: C, 66.92; H, 5.22; N, 5.57. Found: C, 66.87; H, 5.52; N, 5.47.

3-(1*H*-Indol-7-yl)-pro-2-en-1-ol (32)

A solution of **31**[15] (8.0 g, 39.8 mmol) in toluene (60 ml) was added, Dibal-H (1 M intoluene, 40 ml) at −30°C. The reaction mixture was stirred at −30°C for 0.5 h and then quenched with 20 ml of ice water (20 ml) and 3 N HCl (15 ml). After another 0.5 h of stirring, the separated organic layer was condensed. The residue was purified by silica gel chromatography to yield **32** (5.8 g, 84% yield) as yellow oil. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 4.34 (d, J = 5.1 Hz, 2H), 6.32–6.41 (m, 1H), 6.58 (dd, J = 3.0, 2.1 Hz, 1H), 6.85 (d, J = 15.9 Hz, 1H), 7.10–7.16 (m, 2H), 7.23 (d, J = 6.9 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 8.79 (br s, 1H). MS (ESI): 174.1 (M^+ +H); HREIMS: Calculated for $C_{11}H_{11}NO$ (M^+) 173.0841, found 173.0846.

7-(3-Aceoxypropen-1-yl)-1*H*-indole (33)

A solution of **32** (5.8 g, 33.5 mmol) in chloroform (100 ml) containing pyridine (10 ml) was added Ac_2O (5 ml) at 0–10°C. The reaction mixture was stirred at 0–10°C for 5 h and then quenched with 20 ml of ice water (20 ml) and 3 N HCl (20 ml) with stirring. The separated organic layer was washed successively with saturated aqueous $NaHCO_3$ (25 ml) and water (25 ml) and then evaporated. The residue was purified by flash chromatograph (silica, 3/1 (v/v) hexane-ethyl acetate) to yield **33** (6.5 g, 90% yield) as yellow oil. ^{1}H-NMR (500 MHz,$CDCl_3$) δ: 2.12 (s, 3H), 4.80 (d, J = 6.3 Hz, 2H), 6.29–6.40 (m, 1H), 6.57 (dd, J = 3.0, 2.1 Hz, 1H), 6.93 (d, J = 15.9 Hz, 1H), 7.09–7.20 (m, 2H), 7.26 (d, J = 7.8 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 8.62 (br s, 1H). MS (ESI): 216.0 (M^++H); HREIMS: Calculated for $C_{13}H_{13}NO_2$ (M^+) 215.0946, found 215.0951.

2-[3-(1*H*-Indol-7-yl)allyl]malonic acid diethyl ester (34)

A solution of $[(C_6H_5)_3P]_2PdCl_2$ (0.84 g, 1.2 mmol) in THF (30 ml) was added the acetate **33** (6.5 g, 30.2 mmol) in THF (30 ml) by syringe. After a further 0.5 h of stirring, the solution was added to a stirred suspension of sodium malonate (8.2 g, 45.0 mmol) in THF (30 ml). The reaction mixture was stirred at room temperature overnight and then diluted with ethyl acetate (50 ml), and the organic phase washed with saturated aqueous NH_4Cl (40 ml × 2) and water (50 ml). The separated organic layer was condensed. The residue was purified by silica gel chromatography to yield **34** (4.8 g, 45% yield). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.26 (t, J = 7.2 Hz, 6H), 2.84 (t, J = 7.2 Hz, 2H), 3.56 (t, J = 7.2 Hz, 1H), 4.21 (q, J = 7.2 Hz, 4H), 6.16–6.26 (m, 1H), 6.53–6.55 (m, 1H), 6.73 (d, J = 15.9 Hz, 1H), 7.03–7.12 (m, 2H), 7.21–7.23 (m, 1H), 7.52 (d, J = 7.8 Hz, 1H), 8.65 (br s, 1H). MS (ESI): 316.0 (M^++H); HREIMS: Calculated for $C_{18}H_{21}NO_4$ (M^+) 315.1471, found 315.1473.

2-[3-(1*H*-Indol-7-yl)-propyl]propane-1,3-diacetoxymethyl (15)

A solution of the diester **34** (4.8 g, 15.2 mmol) in MeOH (100 ml) was hydrogenated (50 psi, Pd/C) using 10% Pd-C (10 wt%) until completion of reduction (~12 h). The resultant mixture was filtered from Celite and was evaporated to give saturated diester in quantitative yield. The intermediate was taken into ether (16 ml), and added to a suspension of $LiAlH_4$ (2.3 g, 60.6 mmol) in ether (120 ml) at 0°C. After being stirred at 0°C for 10 min and at room temperature for 3 h, the reaction was quenched at 0°C by careful addition of AcOEt (17 ml) followed by MeOH (2 ml) and by 3 N HCl (81 ml). The mixture was extracted with AcOEt, and the organic phase washed with brine and evaporated to dryness to give crude diol (2.0 g). The diol was acetylated (Ac_2O, pyridine) in chloroform by a similar procedure as the acetylation of **32** to yield **15** (3.1 g, 64%). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.40–1.50 (m, 2H), 1.80–2.00 (m, 3H), 2.10 (s, 6H),

2.82 (t, J = 7.8 Hz, 2H), 4.00–4.20 (m, 4H), 6.53–6.55 (m, 1H), 6.96 (d, J = 6.9 Hz, 1H), 7.03 (t, J = 7.2 Hz, 1H), 7.21–7.23 (m, 1H), 7.49 (d, J = 8.1 Hz, 1H), 8.65 (br s, 1H). MS (ESI): 318.0 (M^++H); HREIMS: Calculated for $C_{18}H_{23}NO_4$ (M^+) 317.1627, found 317.1623.

Insulin Receptor Activation Assay

Activation efficacies on cell-based insulin receptor tyrosine kinase were determined using a Chinese hamster ovary (CHO) cell line that overexpresses recombinant human insulin receptor, followed by in vitro determination of receptor tyrosine kinase activity [Zhang et al., 1999; Liu et al., 2000]. Approximately 100,000 CHO cells were used for one assay in each well of a 96-well plate. Cells were incubated overnight and starved in serum free Ham's F-12 medium for 2 h before being stimulated with desired concentration of insulin or test compound for 20 min. Test compounds were predissolved in DMSO as 100 × stocks and serially diluted when needed. Stimulated CHO cells were lysed in 60 µl of lysis buffer; 50 µl of the lysate was transferred to a well on a Flexible Assay Plate precoated with Ab-3 antibody specific to the β-subunit of insulin receptor. The well was then rinsed 3 times and dried before 10 µl of tyrosine kinase reaction mixture (50 mM HEPES, pH 7.4, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mg/ml polyGlu/Tyr and 250,000 cpm γ-P^{33} ATP/well) was added. The reaction was carried out at 25°C for 40 min and terminated by adding 50 µl ice cold 100 mM phosphoric acid. Next, 50 µl of the stopped reaction mixture was transferred to an inch-square P81 paper. The P81 paper was rinsed in Millipore water 5 times to remove leftover radioactive ATP; 5 ml of scintillation cocktail was added to the paper before the count was determined in a liquid scintillation counter.

All measurements were determined in triplicate with blank. Bovine insulin (10 nM) was used as a positive control, to serve as the reference standard. Fifty percentile effective concentration (EC_{50}) was estimated by the dose curve-fitting function in SigmaPlot 8, which models on $Y = \min+(\max-\min)/(1+(X/EC_{50})\text{^Hillslope})$, where Hillslope is Hill coefficient. X represents compound concentration; Y represents observed response, while max and min were approximated automatically by the program during the calculation.

RESULTS AND DISCUSSION

Retroanalysis of Hydroxyfuroic Acid Derivatives

An interesting feature during the synthesis of series 1 compounds is the selective methylation of 3-methoxy-4-phenyl-furan-2,5-dicarboxylic acid to its 5-methyl ester using methyl chloroformate and 4-dimethylaminopyridine (DMAP), in a mixed anhydride method (see section below, Synthesis of the Furoic Acid **9**) [Chou et al., 2006]. Since series 2 compounds share the same hydroxyl furan core, we adapted the same strategy for series 2 compound synthesis, and the retro synthetic disconnection is essentially the same as that of series 1 compound, as illustrated in Figure 2. The desired half ester **C** could be prepared from the furoic diacid **D** by the mixed anhydride method. The series 2 derivatives **A** could be prepared by acylation with a 7-substituted indole **B** with the half ester **C**.

Synthesis of the Furoic Acid 9

Scheme 1 presents the synthetic pathway for the furoic diacid **9**. The furan diester **4** was prepared by adding a mixture of dimethyl diglycolate **1** and *p*-methoxybenzyl (PMB)-protected indolylglyloxalate **2** (1.5:2 molar ratio) to a refluxing suspension of *t*-BuOK (2.7 molar eqs. vs dimethyl diglycolate) in refluxing benzene; the resulting 3-hydroxyfuran intermediates (**3a** and **3b**) were immediately protected by methylation to provide **4** [note: theoretically, one molar equivalent of hydroxide ion would be generated by condensation, leading to partial hydrolysis of the resulting furan diester, but in our setting, executing methylation without purification is advantageous for a yield improvement about 15–20%]. Removal of PMB from **4** would generate **5**, which was achieved by stirring **4** with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in CH_2Cl_2/H_2O 18:1 (v/v) at room temperature [Oikawa et al., 1984]. The deprotected indole **5** was then tosylated to form diester **6**.

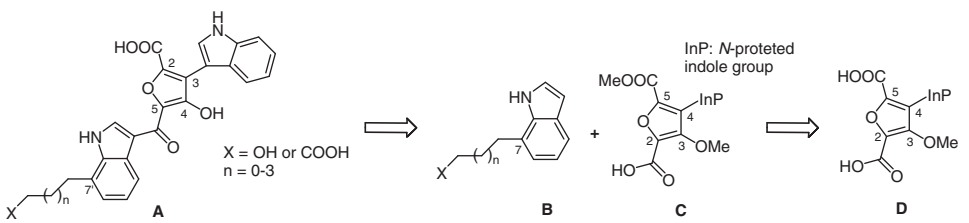
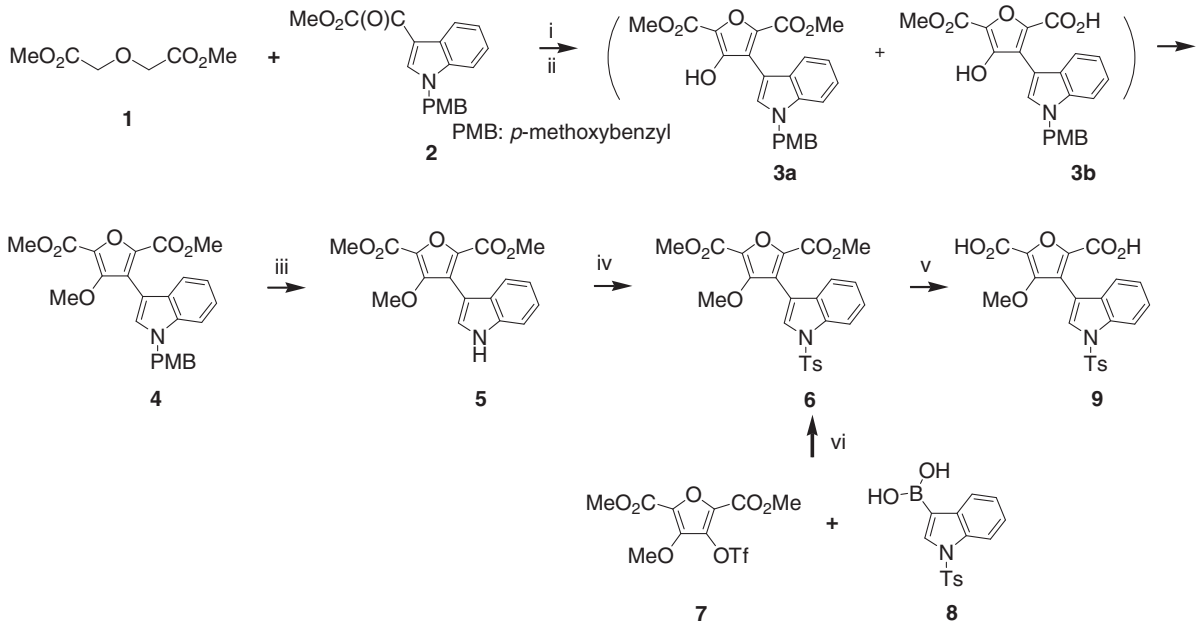
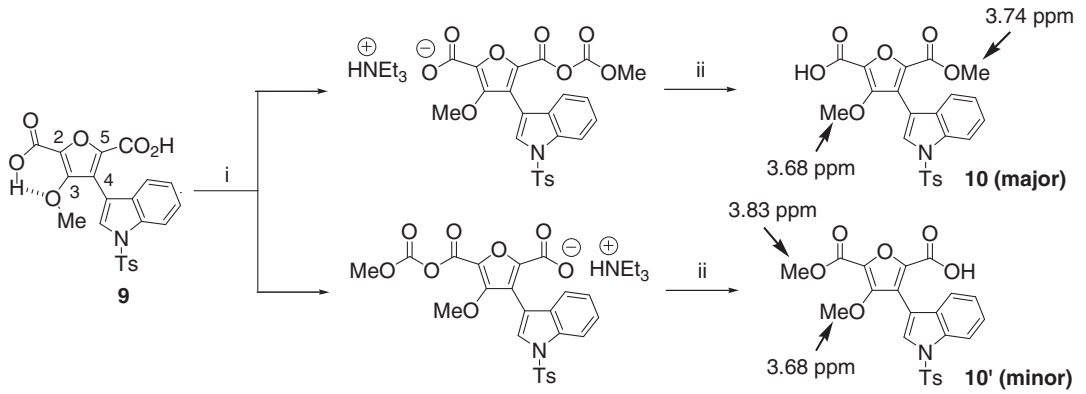


Fig. 2. Retroanalysis of 4-hydroxyfuroic acids.



Scheme 1. Reagents and conditions: (i) K, *t*-BuOH, benzene; (ii) MeI, DMF, 45.3% (two steps); (iii) DDQ, CH_2Cl_2/H_2O (18/1, v/v); 45.5%; (iv) TsCl, 2-butanone, K_2CO_3, reflux, 85.6%; (v) OH^-, MeOH, H_2O, 95%; and (vi) $PdCl_2(PPh_3)_2$ (0.1 eq), Na_2CO_3 (3.0 eq), DMF, 90°C, 12 h, 65.5%.



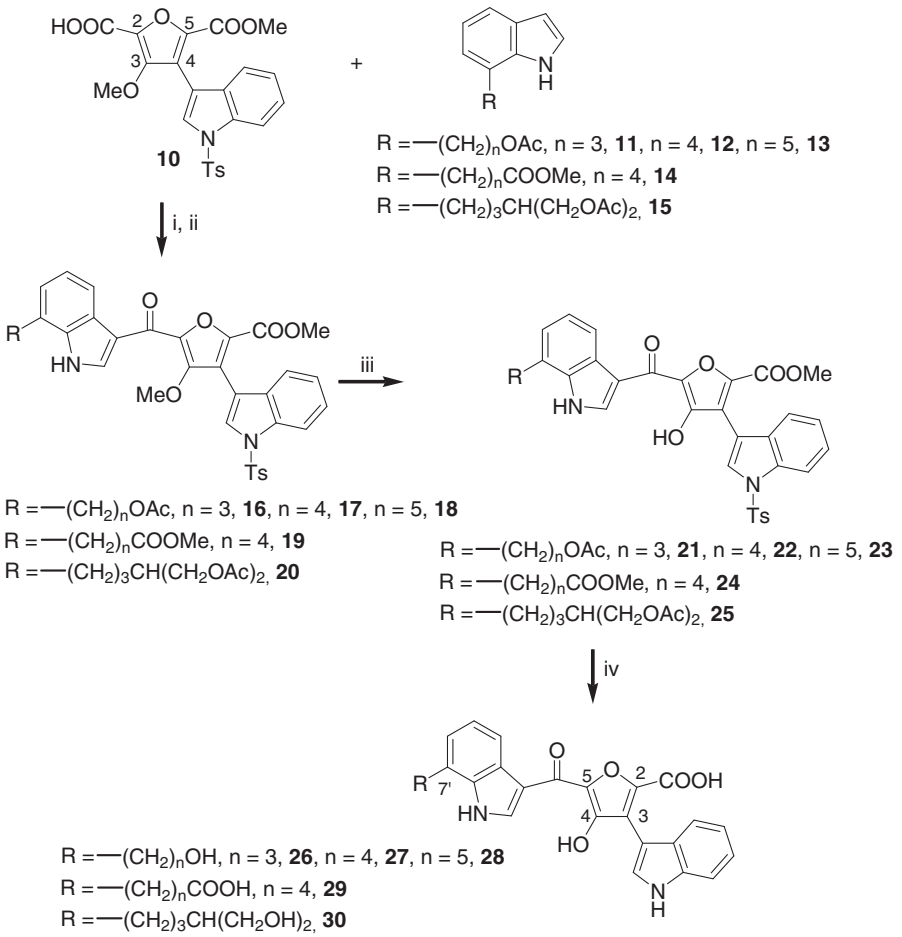
Scheme 2. Reagents and conditions: (i) $ClCO_2Me$ (100 mol%), NEt_3 (107 mol%), CH_2Cl_2 (6 ml/mmol 9) and (ii) DMAP (12 mol%), 65.5% (**10**/**10′** = 7:1 molar ratio).

Alternatively, **6** can be prepared from the metal-catalyzed cross-coupling of the triflate **7** [Chou et al., 2006] with an indolyl boronic acid **8** [Witulski et al., 2000]. Hydrolysis of **6** yielded the diacid **9**. The purpose of tosyl replacement is to avoid undesired electrophilic acylation at the indole ring during the subsequent acylation step. Without the tosyl replacement, the subsequent acylation would have yielded a complicated mixture.

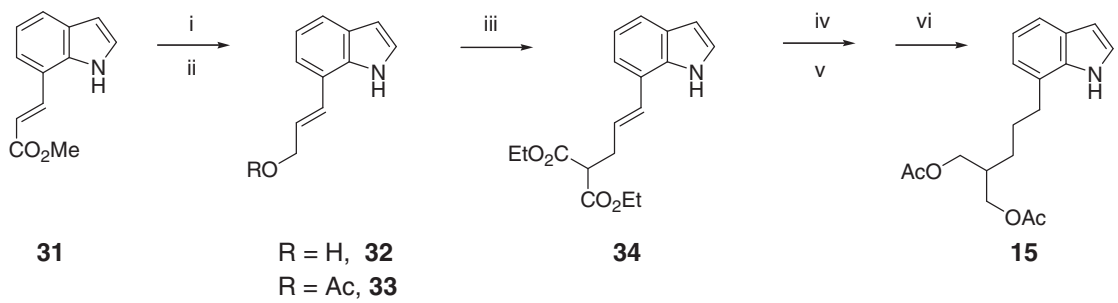
Regioselective Synthesis of the Half Ester 10 From the Diacid 9: Mixed Anhydride Method

The diacid **9** was converted regioselectively into the half ester **10** (diastereomeric ratio: **10**/**10′**∼7/1) by treating it with methyl chloroformate and dichloromethane in the presence of triethylamine and DMAP (Scheme 2). Presumably, the C-2 carboxylic acid of **9** is conjugated with an electron-donating 3-methoxyl group (3-OMe), and the acidic proton is locked intramolecularly by the 3-OMe group, i.e., intramolecular *H*-bonding effect. Therefore, the C-2 carboxylic acid should be less likely to be deprotonated and thus less reactive than C-5 free carboxylic acid when treated with methyl chloroformate and triethylamine. The mixed anhydride intermediate was then decarboxylated to C-5 carboxylic acid methyl ester spontaneously in the presence of a catalytic amount of DMAP [Chou et al., 2006]. As revealed by the ^{1}H-NMR spectrum, the

methyl ester protons of the desired isomer **10** showed a higher chemical shift than **10′** because of the indole ring's shielding effect, and the regiochemistry can also be elucidated by an NOE spectrum. Conversely, the hydrolysis of the diester **6** using a 1.5 M equivalent of LiOH in aqueous methanol yielded the undesired half acid **10′** as the major product (**10**/**10′**∼1/16), indicating that C-5 ester, without intramolecular *H*-bonding, is consistently more reactive than C-2 ester [note: hydrolysis of **6** with 150 mole% of LiOH in aqueous methanol (MeOH: 17.9 ml/mmol **6**, H_2O: 0.9 ml/mmol **6**) at room temperature for 12 h yielded **10′** (undesired), **10** (desired) and intact **6** in 83:5:7 molar ratio].



Scheme 3. Reagents and conditions: (i) oxalyl chloride, DMAP (cat.), room temperature; (ii) 7-substituted indole (**11**–**15**), Et_2AlCl, $CHCl_3$, 51–66%; (iii) BCl_3, CH_2Cl_2, 0°C to room temperature; 80–85%; and (iv) 5% NaOH in methanol, reflux, 66–70%.



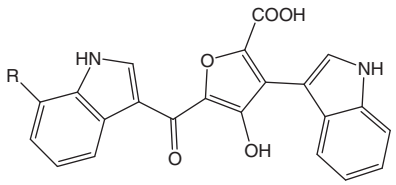
31 **R = H, 32** **R = Ac, 33** **34** **15**

Scheme 4. Reagents and conditions: (i) DIBALH, toluene, 84%; (ii) Ac_2O, pyridine, $CHCl_3$, 86.4%; (iii) $NaCH(COOEt)_2$, $[(C_6H_5)_3P]_2PdCl_2$, THF, 45%; (iv) H_2, Pd-C; (v) $LiAlH_4$, ether; and (vi) Ac_2O, pyridine, $CHCl_3$, 64% (three steps, **34**–**15**).

Hydroxyfuroic Acid Derivatives

The synthetic routes of hydroxyfuroic acids **26–30** are presented in Scheme 3. The acylation of the half ester **10** with 7-substituted indoles **11–15** was performed under mild reaction conditions using diethylaluminum chloride as the catalyst [Okauchi et al., 2000]. The 7-substituted indole intermediates (**11–14**) with a linear carbon chain and a protected hydrophilic end group were prepared by chain extension reactions as described by Chou et al. [2006]. The C-7 dihydroxyalkyl indole intermediate **15**, was prepared from 3-(1*H*-indol-7-yl)acrylic acid methyl ester (**31**) by a six-step sequence, in which the metal-catalyzed coupling (**33**→**34**) is the key step (Scheme 4). The acyl intermediates **16–20** were deprotected under mild reaction conditions using boron trichloride to yield hydroxyfurans **21–25** (see Scheme 3). Finally, simultaneous hydrolysis of the C-2 ester, the C-7′ ester and the *N*-tosyl group under basic condition resulted in the hydroxyfuroic acid derivatives (**26–30**) [Tsai and Chou, 2009].

TABLE 1. Activation of Insulin Receptor by 100 μM of Hydroxyfuroic Acid Compounds



Compound	Side chain R	% Activation[a]	EC_{50} [b]
26	$(CH_2)_2CH_2OH$	15.5±4.9	
27	$(CH_2)_3CH_2OH$	26.0±1.5	
28	$(CH_2)_4CH_2OH$	88.6±7.6	32.9±2.9
29	$(CH_2)_4COOH$	32.6±15.1	
30	$(CH_2)_3CH(CH_2OH)_2$	26.3±13.9	
Compound 2 (Merck)	NA	NA	38.8±4.6

NA, not applicable.

[a]Compound activation is expressed as percentage of activation provided by 10 nM bovine insulin. Values are average±standard deviation (SD) of triplicate measurements.

[b]Fifty percentile effective concentration (EC_{50}) is expressed in μM. EC_{50} of **28** was determined with compound concentrations ranging from 23 to 300 μM, while Compound 2 of Merck ranging from 7.5 to 100 μM. Percentage activation of **28** maximums at 95 μM (48.7% activation), while Compound 2 of Merck maximums at 75 μM (55.3% activation). The EC_{50} parameters are determined by the dose curve fitting function in SigmaPlot 8 with the regression curve of $Y = \min + (\max - \min)/[1 + (x/EC_{50})\text{^Hillslope}]$. Compounds **26**, **27**, **29**, and **30** have too little activation to be measured for EC_{50}.

Biological Evaluations

The 4-hydroxyfuroic acid compounds were subjected to insulin receptor activation assay and the results indicated that cells treated with 100 μM of compounds **26–30** exhibited 12–67% of the receptor tyrosine kinase activity as compared to that offered by 10 nM bovine insulin control (Table 1). Among these compounds, hydroxyfuroic acid **28** is the most potent one. When comparing **28** to Merck's Compound 2, the EC_{50} are 32.9±2.9 μM for **28** and 38.8±4.6 μM for Compound 2, suggesting they have similar potencies at activating insulin receptor tyrosine kinase activity. Figure 3 exhibits dose responses of human insulin receptor to increasing concentrations of compound **A**, **26** and **27**. Compound **A** is a series 1 compound, which has a 3-phenyl instead of 3-indole substitute, showed no appreciable biological activity, whereas **26**, **27** are series 2 compounds and possess substantial insulin receptor activation potential. The responses are normalized against a concurrent insulin receptor activation assay offered by 10 nM bovine insulin stimulation.

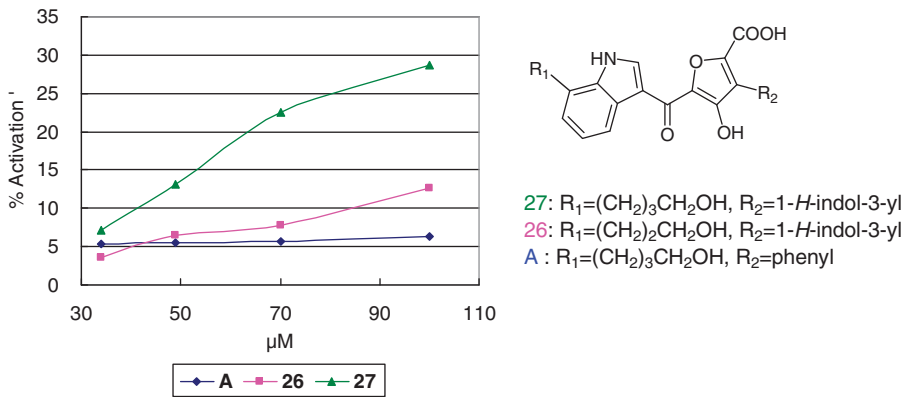


Fig. 3. Dose responses of human insulin receptor to increasing concentrations of compound A of series 1, **26**, and **27** of series 2. The responses are normalized against a concurrent insulin receptor activation assay offered by 10 nM bovine insulin stimulation. [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

CONCLUSION

This work presents an approach of regioselective synthesis for biologically active furandiindole derivatives. The scaffold of these compounds is novel, and this methodology may also be applied to introducing various substituents into the furan ring with desired regiochemistry.

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