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Synthesis and *α*-glycosidase inhibitory evaluation of isatin derivatives

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ABSTRACT

*A series of different substituted isatin derivatives were designed and synthesized about 15-40% overall yields. Three compounds **3a, 3b** and **3c** have not been reported before. Their structures were characterized by ^{1}H and ^{13}C NMR. All the synthesized derivatives were subjected to evaluate theα-glycosidase inhibitory activity. The results indicated that compounds **3a, 3b**and **3d** exhibited α-glycosidase enzyme inhibition activity.*

Keywords: Isatin, *α*-Glycosidase inhibitor, Synthesis

INTRODUCTION

Isatin, an indole derivative, widely presents endogenously in both human and other mammalian tissues and fluids.[1] It was reported that 1-, 3-, 4-, 5-, and 7-substituted isatin derivatives possess a wide range of pharmacological and biological activities, such as anticancer, antibacterial et al.[2-4]

Recently, some literatures reported that isatin compounds exhibited *α*-glycosidase inhibitory activity.[5] In this study, we wish to describe the synthesis and *α*-glycosidase inhibitory evaluation of a series of isatin derivatives.

EXPERIMENTAL SECTION

2.1 Materials and Measurements

All reagents and solvents were purchased from commercially suppliers and used without further purification. 1H NMR spectra were recorded with a Bruker AM-400 NMR spectrometer with CDCl3-d6 or DMSO-d6 as the solvent. 13C NMR spectra were recorded at 100 MHz. All chemical shifts (δH and δC) were reported in parts per million (ppm) and the coupling constants were measured in hertz (Hz). Thin layer chromatography was performed using silica gel 60 F254 plates (Merck) with observation under UV when necessary. Chromatography was performed on 230-400 mesh silica gel.

2.2 Synthesis Route of Isatin Derivatives

2.2.1 Synthesis of Indolin-2-one Derivatives(1a-1e)

A mixture of substituted anilines (0.05mol), hydroxylammonium chloride (0.15mol), Na_2SO_4(0.35mol), hydrochloric acid (5mL, 2mol/L) were stirred in H_2O (250mL) at 95°C. The progress of the reaction was monitored by TLC. After the reaction finished, the reaction mixture was filtered and dried. The crude product was used directly for the next step without further purification.

To a flask (100mL) which contained concentrated sulfuric acid (20mL) added N-2-(hydroxyimino) acetamide derivatives (7.0g) in portions at 50°C with vigorous stirring. The reaction temperature was maintained at 50-75°C. After the addition was completed, the mixture was heated to 80°C and stirred at 80°C for 30 min. The reaction

mixture was cooled to room temperature and then poured onto ice (250g). The solid was filtered out and dried over air to yield the crude which was purified by dissolving in dilute sodium hydroxide (5%, 100mL) followed by acidified with hydrochloric acid (4 mol/L, 20mL). The formed solid was filtered out and dried over air to provide the purified substituted isatin derivatives **1a-1e** (35-71%).

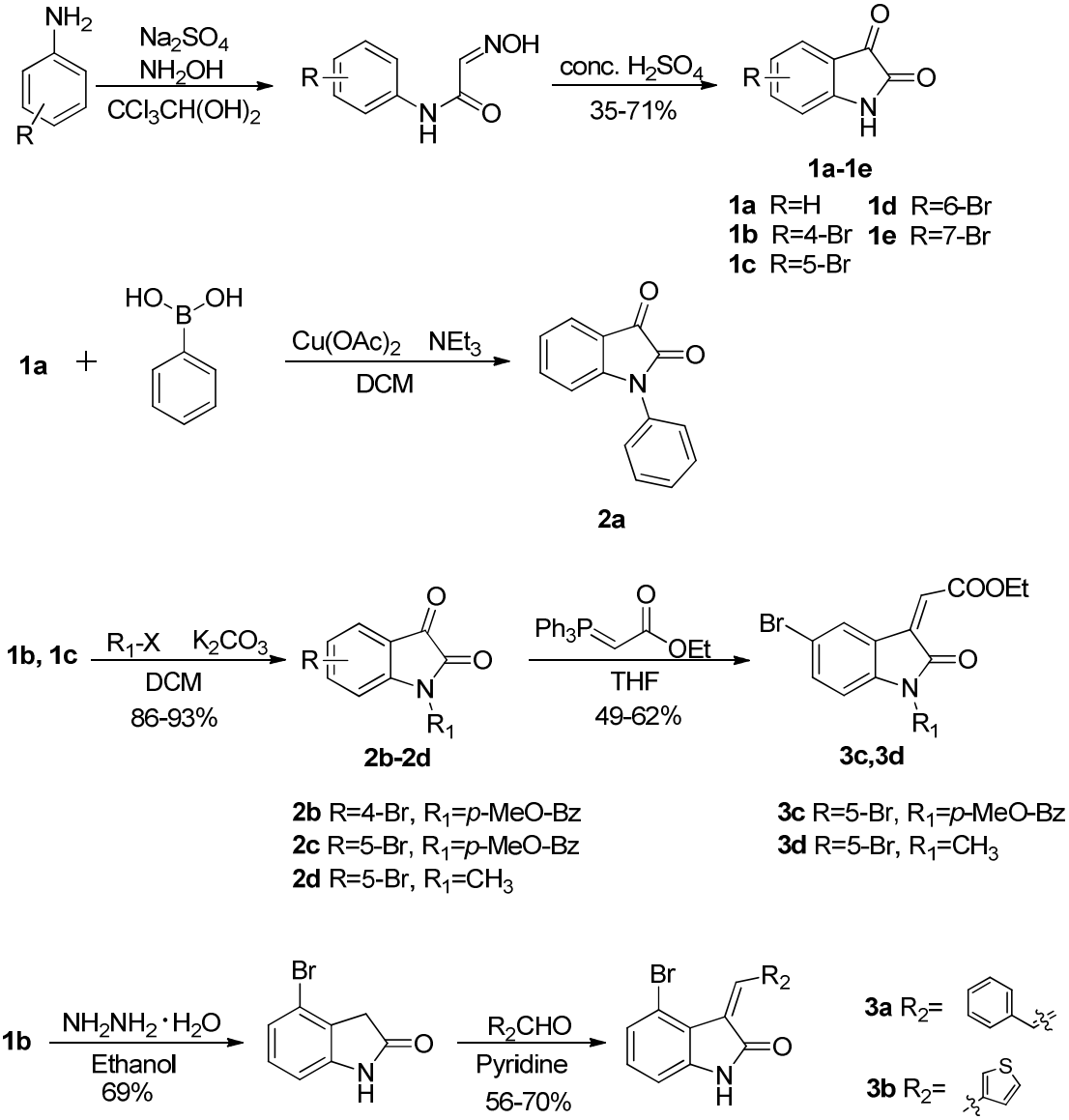


Fig. 1 Synthesis Route of Isatin Derivatives

2.2.2 Synthesis of 1-phenylindoline-2,3-dione(2a)

To a solution of isatin (2g, 13.6mmol) in dry DCM (25mL) was added NEt_3 (5.49g, 54.4mmol) and phenylboronic acid (3.3g,27.2mmol) at 0^oC.The reaction mixture was stirred at room temperature for 24 h. The orange solution was poured into water (25mL) and extracted with DCM (3×100mL). The combined extracts were dried over anhydrous magnesium sulfate, filtered and evaporated. The crude 1-phenylindoline-2,3-dione was purified by flash chromatography with petroleum ether/ethyl acetate (10:1-3:1) to afford product **2a** as a yellow solid. (85%)

2.2.3 Synthesis of N-substituted Isatin Derivatives (2b-2d)

To a solution of 4-bromoindoline-2,3-dione or 5-bromoindoline-2,3-dione (5.10mmol) in dry N,N-Dimethyl formamide (5mL) was added 4-methoxybenzylchloride or CH_3I (10.20mmol) at 0^oC . The reaction mixture was stirred at room temperature for 4 h. The orange solution was poured into water (25mL) and extracted with DCM (3×100mL). The combined extracts were dried over anhydrous magnesium sulfate, filtered and evaporated. The

crude was purified by flash chromatography with petroleum ether/ethyl acetate (8:1) to afford the compounds **2b-2d** (86-93%).

2.2.4 Synthesis of 3-substituted Isatin Derivatives (3a, 3b)

To a solution of 4-bromoindoline-2,3-dione (2g, 8.85mmol) in dry ethanol (25mL) was added hydrazine hydrate (2.21g, 44.2mmol) at 0°C. The reaction mixture was stirred at 78°C for 6 h. The orange solution was poured into water (25mL) and extracted with DCM (3×100mL). The combined extracts were dried over anhydrous magnesium sulfate, filtered and evaporated. The crude was purified by flash chromatography with petroleum ether/ethyl acetate 10:1-3:1 to afford 4-bromoindolin-2-one.

To a solution of 4-bromoindolin-2-one (0.5g, 8.85mmol) in dry ethanol (25mL) was added pyridine (75mg, 0.89mmol) and cinnamaldehyde or 2-thenaldehyde (10.2mmol) at 0°C. The mixture stirred at 78°C for 6 h. The orange solution was poured into water (25mL) and extracted with DCM (3×100mL). The combined extracts were dried over anhydrous magnesium sulfate, filtered and evaporated. The crude was purified by flash chromatography with petroleum ether/ethyl acetate 10:1-3:1 to afford **3a** and **3b** (56-70%).

2.2.5 Synthesis of 3-substituted Isatin Derivatives (3c, 3d)

To a solution of compounds **2c** or **2d** (0.30mmol) in dry THF (10mL) was added (carbethoxymethylene) triphenylphosphorane (0.45mmol) at 0°C. The reaction mixture was stirred under reflux for 3 h. The orange solution was poured into water (25mL) and extracted with DCM (3×50mL). The combined extracts were dried over anhydrous magnesium sulfate, filtered and evaporated. The crude was purified by flash chromatography with petroleum ether/ethyl acetate 10:1-1:1 to afford compounds **3c**and **3d** (49-62%).

2.3 Biological Assay

The commercially available α-glucosidase from baker's yeast (Sigma, G5003) was selected as the target protein using p-nitrophenyl-α-D-glucopyranoside (pNGP, Sigma, N1377) as the substrate. The compounds and acarbose were dissolved in DMSO. The enzyme and the substrate were dissolved in 0.05M potassium phosphate buffer with pH6.8. The enzymatic reaction mixture composed of 20μL α-glucosidase (0.06U), 30 μL of 0.5 mM substrate, 10 μL of test compound and 140 μL of potassium phosphate buffer was incubated at 37°C for 30 min. The enzymatic activity was detected by spectrophotometer at the wavelength of 405 nm. Results are the average of three independent experiments and each performed in duplicate.

RESULTS AND DISCUSSION

3.1 Characterize Isatin Derivatives by ^{1}H and ^{13}C NMR.

3.1.1 Indoline-2,3-dione (1a)

^{1}H NMR (400 MHz, DMSO-d_6): 6.90 (d, *J*=8.0 Hz, 1H), 7.06 (t, *J*=14.8 Hz, 1H),7.49(d, *J*=8.0 Hz, 1H),7.58 (t, *J*=14.8 Hz, 1H),11.04(s, 1H).

3.1.2 4-bromoindoline-2,3-dione (1b)

^{1}H NMR (400 MHz, DMSO-d_6): 6.89 (d, *J*=8.0 Hz, 1H), 7.23(d, *J*=8.0 Hz, 1H), 7.46(t, *J*=16.0 Hz, 1H), 11.19(s, 1H).

3.1.3 5-bromoindoline-2,3-dione (1c)

^{1}H NMR (400 MHz, DMSO-d_6): 6.87(d, *J*=8.4 Hz, 1H), 7.66 (s, 1H), 7.74 (d, *J*=8.4 Hz, 1H), 11.15(s, 1H).

3.1.4 6-bromoindoline-2,3-dione (1d)

^{1}H NMR (400 MHz, DMSO-d_6): 7.08 (s, 1H), 7.26 (d, *J*=8.0 Hz, 1H), 7.44 (d, *J*=8.0 Hz, 1H), 11.15 (s, 1H).

3.1.5 7-bromoindoline-2,3-dione (1e)

^{1}H NMR (400 MHz, DMSO-d_6): 6.95 (t, *J*=15.6 Hz, 1H), 7.44 (d, *J*=7.6 Hz, 1H), 7.72(d, *J*=7.6 Hz, 1H), 11.26(s, 1H).

3.1.6 1-phenylindoline-2,3-dione (2a)

^{1}H NMR (400 MHz, DMSO-d_6): 6.82(d, *J*=8.0 Hz, 1H), 7.18 (d, *J*=8.0 Hz, 1H), 7.21-7.51(m, 3H), 7.59-7.63(m, 3H), 7.66(d, *J*=8.0 Hz, 1H).

3.1.7 4-bromo-1-(4-methoxybenzyl) indoline-2,3-dione (2b)

^{1}H NMR (400 MHz, $CDCl_3$): 3.78 (s, 3H), 4.86 (s, 2H), 6.75(d, *J*=8.0 Hz, 1H), 6.86 (d, *J*=8.4 Hz, 2H), 7.19-7.31(m,

4H). ^{13}C NMR (100 MHz, $CDCl_3$): 43.5, 55.3, 109.8, 114.4, 114.4, 116.4, 121.5, 126.1, 128.4, 128.8, 128.8, 138.3, 152.2, 157.3, 159.5, 180.7.

3.1.8 5-bromo-1-(4-methoxybenzyl) indoline-2,3-dione (2c)

^{1}H NMR (400 MHz, $CDCl_3$): 3.78 (s, 3H), 4.85 (s, 2H), 6.69 (d, *J*=8.4 Hz, 1H), 6.87(d, *J*=8.8 Hz, 2H), 7.24 (d, *J*=8.8 Hz, 2H), 7.58 (d, *J*=8.4 Hz, 1H), 7.70 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 43.6, 55.3, 112.7, 114.5, 114.5, 116.7, 118.8, 125.9, 128.1, 128.9, 128.9, 140.4, 149.4, 157.5, 159.5, 182.2.

3.1.9 (Z)-4-bromo-3-((E)-3-phenylallylidene) indolin-2-one(3a)

^{1}H NMR (400 MHz, $CDCl_3$): 6.85(d, *J*=8.0 Hz, 1H), 7.09-7.18(m, 3H), 7.31-7.47(m, 4H), 7.62 (d, *J*=8.0 Hz, 2H), 8.27 (d, *J*=8.8 Hz, 1H), 8.65-8.68(m, 1H), 10.84(s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 110.3, 112.7, 114.7, 114.7, 117.1, 118.2, 125.2, 125.9, 128.4, 128.8, 128.8, 132.2, 135.7, 138.2, 140.4, 148.6, 166.3.

3.1.10 (Z)-4-bromo-3-(thiophen-2-ylmethylene) indolin-2-one(3b)

^{1}H NMR (400 MHz, DMSO-d_6): 6.80-6.81(m, 1H), 6.89(d, *J*=7.2 Hz, 1H), 7.14 (t, *J*=15.6 Hz, 1H), 7.21 (d, *J*=8.0 Hz, 1H), 8.03(s, 1H), 8.36(s, 1H), 8.47(s, 1H), 10.92(s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): 109.4, 114.4, 116.1, 120.9, 121.0, 122.4, 124.2, 126.5, 130.2, 143.3, 147.6, 150.5, 166.8.

3.1.11 (Z)-ethyl 2-(5-bromo-1-(4-methoxybenzyl)-2-oxoindolin-3-ylidene) acetate (3c)

^{1}H NMR (400 MHz, $CDCl_3$): 1.38 (t, *J*=14.4 Hz, 1H), 3.76 (s, 3H), 4.32-4.37(m, 2H), 4.85(s, 2H), 6.58 (d, *J*=8.4 Hz, 1H), 6.83 (d, *J*=8.8 Hz, 2H), 6.99 (s, 1H), 7.19 (d, *J*=8.8 Hz, 2H), 7.36 (d, *J*=8.4 Hz, 1H), 8.72(s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 14.1, 43.4, 55.2, 61.5, 110.5, 114.3, 114.3, 115.5, 121.6, 124.2, 126.9, 128.6, 128.6, 131.6, 134.8, 136.6, 143.9, 159.2, 165.3, 167.0.

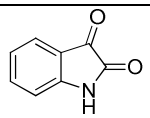
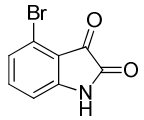
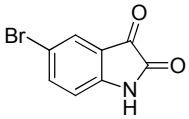
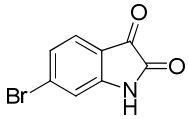
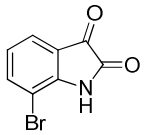
3.1.12 (Z)-ethyl 2-(5-bromo-1-methyl-2-oxoindolin-3-ylidene) acetate(3d)

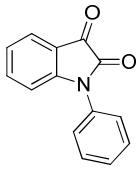
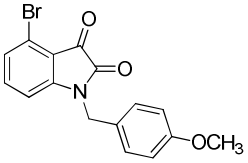
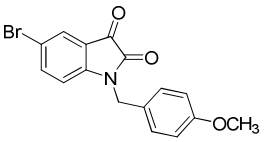
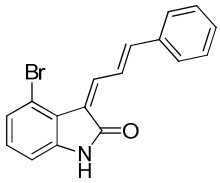
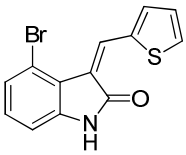
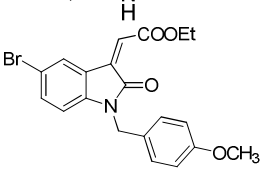
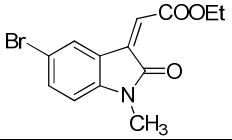
^{1}H NMR (400 MHz, $CDCl_3$): 1.38 (t, *J*=14.4 Hz, 3H), 3.20 (s, 3H), 4.31-4.37 (m, 2H), 6.66 (d, *J*=8.4 Hz, 1H), 6.90(s, 1H), 7.47 (d, *J*=8.0 Hz, 1H), 8.70(s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 14.1, 26.3, 61.4, 109.4, 115.4, 121.3, 123.9, 131.5, 134.8, 136.7, 144.8, 165.2, 166.9.

3.2 *α*-glycosidase Enzyme Inhibition Activity Assay

All the above compounds were evaluated for their α-glucosidase inhibitory activity at 50μM and 5μM. As showed in Table 1, compounds **1a-1e** and **2a-2c** have no the *α*-glycosidase inhibitory activity. However, **3a,3b** and **3d** showed better *α*-glycosidase inhibitory activity, especially, compound **3b**. The results suggested that the modification at C-3 could improve the *α*-glycosidase inhibitory activity, but a bulky N-substituted group is not favorable, such as compound **3c**.

Table 1. Inhibition Activity of Isatin Derivatives

Compd.	Structure	Inhibition (%)	
		5 μM	50 μM
1a		NA[a]	NA
1b		NA	8.10 ± 14.16
1c		NA	NA
1d		NA	NA
1e		NA	NA

2a		NA	NA
2b		NA	NA
2c		NA	NA
3a		NA	50.63 ± 8.75
3b		NA	100.82 ± 19.05
3c		NA	NA
3d		NA	9.92±5.89

[a]NA=no activity

CONCLUSION

A series of N-1 and C-3 substituted isatin derivatives were synthesized and tested for*α*-glycosidase inhibitory activity. The study of these compounds led to the identification of a new isatin**3b**as potent*α*-glycosidase inhibitor. Further structural modification of **3b**is ongoing in our laboratory.

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