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Synthesis and Bioactivity Evaluation of 2-Arylbenzimidazole Analogues

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Benzimidazole is an important drug intermediate. Benzimidazole analogues have shown antibacterial, antiviral, resisting parasites medicinal activity. In this paper, we used phenylenediamine as a starting material through cyclization, acylation reaction to synthetize 2-arybenzimidazole analogues. Twenty-two analogues were synthesized and characterized on the basis of ^{1}H NMR spectra. All this compounds were tested through novel yeast-based screening method to evaluate their bioactivities.

Keywords: Synthesis, 2-Arybenzimidazole, Yeast-based screening.

INTRODUCTION

Benzimidazole derivatives with their unique biological activity[1], such as sterilization, antiinflammatory, antioxidant activity are widely used as many important pharmaceutical intermediates[2-5]. In this paper, we mainly study the bioactivity of 2-arybenzimidazole analogues as SIRT1 activators. SIRT1, an NAD^+-dependent sirtuin deacetylase, has emerged as potential therapeutic targets for treatment of human illnesses such as type II diabetes[6,7], cardiovascular[8] and neurodegenerative diseases[9]. SRT1720 (Fig. 1), an imidazothiazole derivative, recently made as the most potent SIRT1 activator is structurally unrelated to resveratrol[10]. In yeast, sir2 is the closet homolog of SIRT1[11]. A mechanistic explanation has been proposed whereby calorie restriction slows aging by activating Sir2[12]. Over-expression of Sir2 increases life span by 30-40 %[13,14]. According to report that using sir2 activators can increase the yeast replicative lifespan and reduce chronological lifespan, the characterization is their gradient of growth curves increased[15-18]. On this basis, we report a novel yeast-based screening method to detect the Sirt1 activators. The aim of this work is to quickly screen lower biology toxic and new scaffold structure for novel potent SIRT1 activators.

EXPERIMENTAL

All reagents and solvents used were of reagent grade. Reaction temperatures were controlled by oil bath temperature modulator. Thin layer chromatography (TLC) was performed using E. Merck silica gel 60 GF254 precoated plates (0.25 mm). Silica gel (particle size 200-400 mesh) was used for flash chromatography. 1H spectra were recorded on Bruker AM-400 NMR spectrometer in deuterated chloroform and deuterated DMSO.

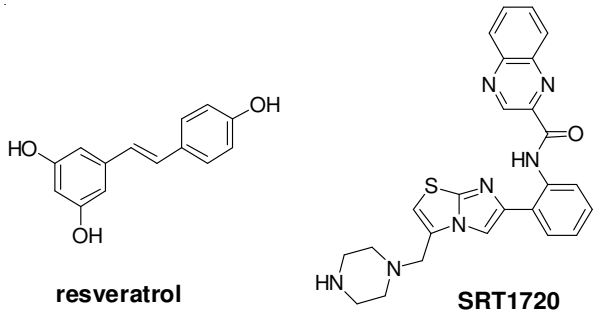
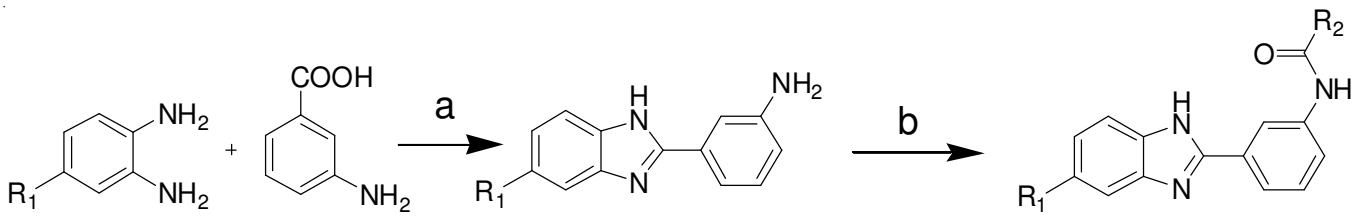


Fig. 1. Structures of the reported SIRT1 activators

Synthsis route of the target compounds (Scheme-I)

First step (a) : To a flask 1,2-diaminobenzene (3.24 g, 30 mmol), 3-aminobenzoic acid (4.93 g, 36 mmol) and PPA (33 g) were added, then the reaction mixture was allowed to be heated to 200 °C by oil bath and kept reflux for 5 h. After the reaction was completely finished, the reaction mixture was slowly poured into ice water and the resulting mixture was basified with solid NaOH and $NaHCO_3$. At pH 8-10, the precipitate was filtered, washed with water and dried to obtain the crude product, which purified by flash column chromatography (silica gel, pure CH_2Cl_2) to afford intermediate.

Second step (b): In a round-bottomed flask, aromatic carboxylic acid (1.2 mmol) was dissolved in suitable amount of CH_2Cl_2 (25 mL), followed by adding ethylenediamine



Scheme-I: Reagents and conditions: (a) PPA, 200 °C, 6 h (yield 85-93 %); (b) RCOOH,CH_2Cl_2, EDCI, DMAP, Et_3N, 60 °C, 6 h (yield 32-86 %)

chloride (EDCl) (1.5 mmol), Et_3N (2 mmol), DMAP (0.5 mmol) and intermediate 1 (1 mmol). Then the reaction mixture was allowed to heat at 80 °C and kept reflux for 6 h. After cooling the reaction mixture was poured into a 100 mL separating funnel, and water was added, then extracted by CH_2Cl_2, the organic layer was washed by water, saturated salt water and dried by sodium sulfate. The organic phase was evaporated under vacuum to remove the CH_2Cl_2, providing the crude product. Then it was purified by flash column chromatography to afford the target compounds **1-22**.

Synthesis of the target compounds 1-22 is outlined in Table-1

^{1}H NMR data of compounds 1-22

Compound **1**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.41 (t, 1H, *J* = 4.2 Hz), 8.08 (s, 1H), 7.88-7.86 (m, 3H), 7.65-7.63 (m, 3H), 7.59-7.56 (m, 1H), 7.52-7.48 (m, 2H), 7.43 (t, 1H, *J* = 7.8 Hz), 7.29-7.25 (m, 3H).

Compound **2**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 10.45 (s, 1H), 8.48 (s, 1H), 8.01 (s, 1H), 7.92 (d, 1H, *J* = 6.4 Hz), 7.88 (d, 2H, *J* = 8.8 Hz), 7.82 (s, 1H), 7.56 (s, 1H), 7.51 (d, 2H, *J* = 12.8 Hz), 7.26 (s, 2H), 7.01 (d, 2H, *J* = 9.2 Hz), 3.90 (s, 3H).

Compound **3**: ^{1}H NMR (400 MHz DMSO): δ (ppm) 12.76 (d, 1H, *J* = 15.2 Hz), 10.43 (s, 1H), 8.68 (s, 1H), 8.03 (d, 2H, *J* = 7.2 Hz), 7.86-7.84 (m, 2H), 7.64-7.31 (m, 6H), 7.06-7.01 (m, 1H), 2.44-2.43 (m, 3H).

Compound **4**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.39 (s, 1H), 7.99 (s, 1H), 7.87-7.84 (m, 3H), 7.62 (d, 1H, *J* = 8 Hz), 7.54 (d, 1H, *J* = 8 Hz), 7.44 (t, 1H, *J* = 8 Hz), 7.40 (s, 1H), 7.10 (d, 1H, *J* = 8.4 Hz), 6.99 (d, 2H, *J* = 8.8 Hz), 3.89 (s, 3H), 2.49 (s, 3H).

Compound **5**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.34 (s, 1H), 8.11 (s, 1H), 7.81 (d, 1H, *J* = 8 Hz), 7.71-7.67 (m, 2H), 7.38 (t, 1H, *J* = 8 Hz), 7.25-7.24 (m, 1H), 6.96 (d, 2H, *J* = 2 Hz), 6.60 (t, 1H, *J* = 2.2 Hz), 3.80 (s, 6H).

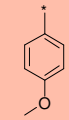
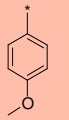
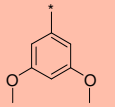
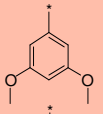
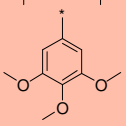
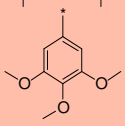
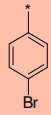
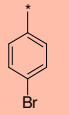
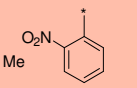
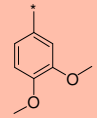
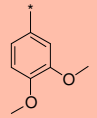
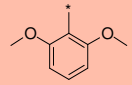
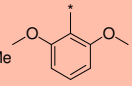
Compound **6**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.40 (t, 1H, *J* = 1.8 Hz), 8.02 (s, 1H), 7.87 (d, 1H, *J* = 8 Hz), 7.66-7.63 (m, 1H), 7.55 (s, 1H), 7.47 (t, 2H, *J* = 8 Hz), 7.41 (s, 1H), 7.12-7.09 (m, 1H), 6.99 (d, 2H, *J* = 2.4 Hz), 6.65 (t, 1H, *J* = 2.2 Hz), 3.86 (s, 6H), 2.49 (s, 3H).

Compound **7**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.77 (s, 1H), 8.27 (s, 1H), 7.71 (t, 2H, *J* = 6.8 Hz), 7.55-7.53 (m, 2H), 7.28 (s, 1H), 7.25 (t, 1H, *J* = 7.8 Hz), 7.22-7.20 (m, 2H), 3.87 (s, 3H), 3.75 (s, 6H).

Compound **8**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.34 (t, 1H, *J* = 1.8 Hz), 8.07 (s, 1H), 8.82-7.77 (m, 2H), 7.55 (s, 1H), 7.48 (t, 2H, *J* = 8 Hz), 7.40 (s, 1H), 7.11 (s, 3H), 3.94 (s, 6H), 3.93 (s, 3H), 2.49 (s, 3H).

Compound **9**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 9.88 (s, 1H), 8.46 (s, 1H), 7.90 (d, 1H, *J* = 7.6 Hz), 7.81 (s, 1H), 7.65 (d, 2H, *J* = 2.4 Hz), 7.51-7.49 (m, 3H), 7.39 (s, 1H), 7.30-7.27 (m, 4H), 2.53 (s, 3H).

TABLE-1
STRUCTURES AND YIELDS OF TARGET COMPOUNDS

Compound	R^1	R^2	Yield	Compound	R^1	R^2	Yield
1	H		62.2%	2	Me		48.2%
3	H		59.4%	4	Me		53.4%
5	H		45.8%	6	Me		35.9%
7	H		72.6%	8	Me		42.9%
9	H		47.9%	10	Me		63.9%
11	H		86.1%	12	Me		43.1%
13	H		35.1%	14	Me		56.1%
15	H		55.3%	16	Me		60.7%
17	H		40.6%	18	Me		39.6%
19	H		40.3%	20	Me		53.3%
21	H	H_3C—*	32.3%	22	Me	H_3C—*	48.3%

Compound **10**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.75-8.74 (m, 2H), 8.47-8.45 (m, 2H), 8.41-8.39 (m, 2H), 7.64-7.60 (m, 2H), 7.47 (t, 1H, *J* = 4.2 Hz), 7.45 (s, 1H), 7.43 (s, 1H), 7.41 (d, 1H, *J* = 0,4 Hz), 7.39 (s, 1H), 2.68 (s, 6H).

Compound **11**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.41 (t, 1H, *J* = 2 Hz), 7.92 (s, 1H), 7.86 (d, 1H, *J* = 7.6 Hz), 7.78-7.75 (m, 3H), 7.68-7.66 (m, 2H), 7.52 (t, 1H, *J* = 8 Hz), 7.32-7.26 (m, 3H).

Compound **12**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.35 (s, 1H), 8.12 (s, 1H), 7.86-7.81 (m, 3H), 7.61-7.60 (m, 1H), 7.59-7.51 (m, 2H), 7.48 (t, 2H, *J* = 7.4 Hz), 7.38 (t, 2H, *J* = 7.8 Hz), 7.09-7.07 (m, 1H), 2.46 (s, 3H).

Compound **13**: ^{1}H NMR (400 MHz DMSO): δ (ppm) 12.95 (s, 1H), 10.79 (s, 1H), 8.88 (t, 1H, *J* = 1.8 Hz), 8.71 (t, 1H, *J* = 1.6 Hz), 8.49-8.46 (m, 2H), 7.94-7.86 (m, 3H), 7.67 (d, 1H, *J* = 7.2 Hz), 7.60-7.54 (m, 2H), 7.24-7.20 (m, 2H).

Compound **14**: ^{1}H NMR (400 MHz DMSO): δ (ppm) 12.79 (d, 1H, *J* = 16 Hz), 10.78 (s, 1H), 8.88 (d, 1H, *J* = 1.2 Hz), 8.68 (s, 1H), 8.48-8.46 (m, 2H), 7.94-7.86 (m, 3H), 7.58-7.32 (m, 3H), 7.07-7.01 (m, 1H), 2.44 (d, 3H, *J* = 7.6 Hz).

Compound **15**: ^{1}H NMR (400 MHz DMSO): δ (ppm) 12.94 (s, 1H), 10.85 (s, 1H), 8.64 (s, 1H), 8.19-8.17 (m, 1H), 7.91-7.88 (m, 2H), 7.84-7.76 (m, 2H), 7.68 (t, 2H, *J* = 8.8 Hz), 7.54 (t, 2H, *J* = 8 Hz), 7.25-7.17 (m, 2H).

Compound **16**: ^{1}H NMR (400 MHz DMSO): δ (ppm) 11.06 (s, 1H), 8.69 (s, 1H), 8.20 (d, 1H, *J* = 8 Hz), 7.96-7.91 (m, 2H), 7.83(t, 2H, *J* = 6.4 Hz), 7.79-7.71 (m, 2H), 7.68 (d, 2H, *J* = 10.8 Hz), 7.58 (s, 1H), 7.33 (d, 1H, *J* = 7.6 Hz), 3.17 (s, 3H).

Compound **17**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.42 (s, 1H), 7.99 (s, 1H), 7.86 (d, 1H, *J* = 8 Hz), 7.73 (d, 1H, *J* = 8 Hz), 7.52-7.48 (m, 2H), 7.44-7.41 (m, 1H), 7.30-7.26 (m, 5H), 6.94 (d, 1H, *J* = 8 Hz), 3.97 (s, 6H).

Compound **18**: ^{1}H NMR (400 MHz DMSO): δ (ppm) 10.76 (s, 1H), 8.02 (s, 1H), 7.99 (s, 1H), 7.73 (t, 3H, *J* = 9.8 Hz), 7.61 (s, 1H), 7.55 (t, 1H, *J* = 8 Hz), 7.36 (t, 4H, *J* = 8.6 Hz), 2.51 (s, 6H), 2.33 (s, 3H).

Compound **19**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 10.56 (s, 1H), 8.44 (s, 1H), 8.02 (s, 1H), 7.87 (d, 1H, *J* = 8 Hz), 7.58 (d, 3H, *J* = 8 Hz), 7.36 (t, 1H, *J* = 8 Hz), 7.28-7.22 (m, 3H), 6.51 (d, 2H, *J* = 8.4 Hz), 3.75 (s, 6H).

Compound **20**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.55 (s, 1H), 8.31 (s, 1H), 7.77-7.67 (m, 2H), 7.44 (s, 1H), 7.26-7.16 (m, 3H), 7.00 (d, 1H, *J* = 8 Hz), 6.38 (s, 2H), 3.62-3.49 (m, 6H), 2.42 (s, 3H).

Compound **21**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.33 (s, 1H), 7.88 (s, 1H), 7.64 (s, 2H), 7.44 (d, 3H, *J* = 4.4 Hz), 7.29-7.26 (m, 3H), 2.22 (s, 3H).

Compound **22**: ^{1}H NMR (400 MHz $CDCl_3$): δ (ppm) 8.27 (s, 1H), 7.83 (d, 1H, *J* = 6.8 Hz), 7.53 (d, 2H, *J* = 9.6 Hz), 7.45-7.411 (m, 4H), 7.09 (d, 1H, *J* = 8 Hz), 2.48 (s, 3H), 2.20 (s, 3H).

Yeast strains and culture condition: Yeast strains used throughout this study were Yeast Parental strain-BY4743 (Sir2) ordered from Thermoscientific, USA. All yeast cells were grown at 30 °C. The yeast strains were grown on YPD (yeast peptone dextrose) medium (1 % yeast extract, 2 % peptone, 2 % glucose). All components were of analytical quality. All compounds tested were dissolved in DMSO. Drug screening assay was determined in yeast peptone dextrose medium.

Growth curve of the yeast: A Bioscreen C MBR machine (Growth Curves USA, Piscataway, NJ) was used for all outgrowth assays. For outgrowth of aged cells, 5 µL of the aging culture was inoculated into 145 µL of rich yeast peptone dextrose medium in a Bioscreen Honeycomb 100-well plate (cat no. 9502550). Compounds were added to corresponding number of tubes to a final concentration of 100 µM/L. Incubation of the plate was kept constant at 30 °C, with the shaking module set to high continuous shaking. Absorbance readings at 600 nm (wideband range) were taken every 0.5 h for 24 h. OD data were normalized for background prior to presentation by subtracting the initial OD value at t = 0 from each subsequent OD reading. The concentration of DMSO in the growth media was kept below 0.1 %, which had no detectable effect on yeast growth. Growth curves were displayed with Excel/Growth Curves software.

RESULTS AND DISCUSSION

In step (a) we used PPA to catalyze carboxylic acid compounds and *o*-phenylendiamine reaction. We optimized and confirmed 200 °C, 6h and n(benzoic acid): n(diaminobenzene) = 1.2:1 are the best reaction conditions, the yield increased about more 15-20 % than other methods[19-21]. This method has many advantages, such as lower cost, few side effect and easier purification.

In step (b), a majority of target compounds can be obtained through EDCI, DMAP catalyzing acylation reaction. However, compound **15**, **16**, **19**, **20** had larger steric hindrance and did not react. We changed the method and chose TsCl and Py catalyze reaction for making **15**, **16**, **19**, **20**, the yield was 40-60 %.

Yeast-based drug screening assay: Yeast as model of drug screening for SIRT1 activators is based on the homologue between yeast Sir2 and human SIRT1[11]. Here we used the Bioscreen CMBR machine for measuring yeast growth curves by monitoring outgrowth of yeast cells[22]. The results showed that this method provides growth curves with decreased variability comparable to traditional one. If compounds can activate Sir2 leading to increase replicative life span and reduce chronological lifespan, which could have higher gradient of growth curves than blank yeast[15-18].

Primary SAR analysis outlined based on Fig. 2 as follows: Based on growth curves, many compounds have distinct effect on the growth curve of yeast. Compared with resveratrol and blank, the curve show that resveratrol could promote the proliferation of yeast, but has no effect on the final biomass and implied no cytotoxicity on yeast. Most of the compounds had inhibition on the growth of yeast, showing that there were some cytotoxicities. Among them,compounds **6**, **11**, **14** had obvious inhibitory effects on yeast growth and may have stronger cytotoxicities. Fortunately, the data showed that compounds **2** have higher activity than resveratrol and non-cytotoxicity.

The result of the gradient of growth curves[22] is outlined on Fig. 3. Gradient = (OD12 h-OD4 h) / (t12-t4), OD12 h and OD4 h indicate the absorption of yeast growth after 12 h and 4 h, respectively..

Base on the gradient of growth curves, *para*-methoxyl group at benzene has the best activity, and the more the number of methoxyl group the less the activity is. In addition, wherever two methoxyl groups are on beneze ring, the activity didn't change obviously. *Ortho*-nitro group at benzene ring also had better effect than *meta*-position. According to effects of **1-22** on Yeast strain date, methyl group at benzene may be an active group, because compounds **2** and **4** have the most active property for activating Sir2 yeast, but compound **2** has a little cytotoxicity on yeast. In all, we obtained one lead compound **4** based on above analysis.

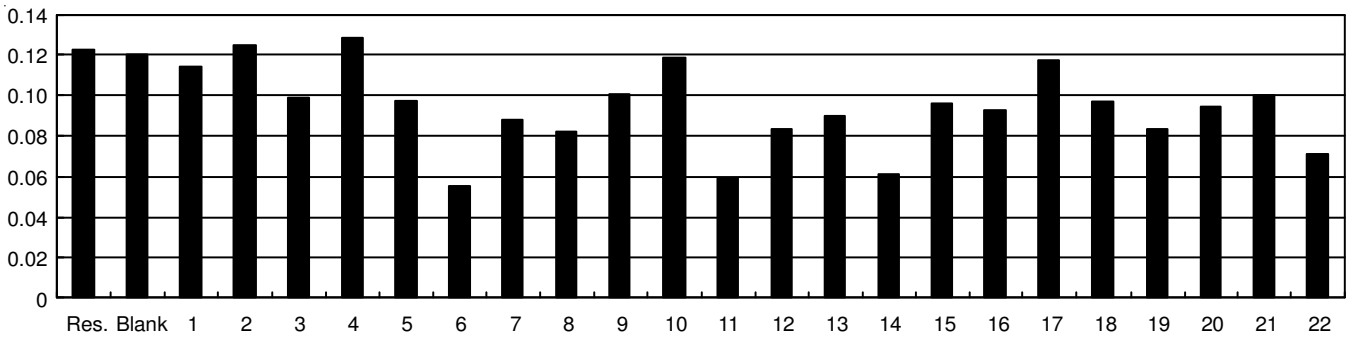


Fig. 3. Gradient of growth curves of yeast with compounds (100 μM L^{-1})

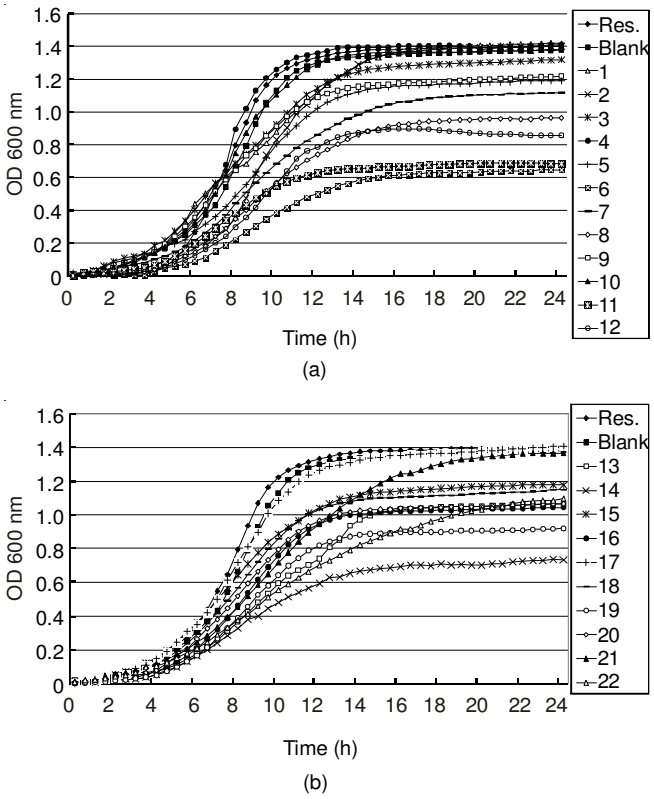


Fig. 2. Effect of all compounds concentration (100 μM L^{-1}) on the growth of yeast

Conclusion

In summary, we made a series of compounds seeking the leads for activating Sirt1. The whole experimental results are encouraging and one lead compound for potential SIRT1 activator had been screened out. In the further research, the activity of compound **4** *in vivo* will be tested in our lab.

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