Indo. J. Chem., 2012, 12 (2), 172 - 177

IN VITRO ACTIVITY AND COMPARATIVE STUDIES OF SOME ORGANOTIN(IV) BENZOATE DERIVATIVES AGAINST LEUKEMIA CANCER CELL, L-1210

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Received November 28, 2011; Accepted February 27, 2012

ABSTRACT

A series of dibutyl-, diphenyl- and triphenyltin(IV) benzoate derivatives has been prepared. The products were obtained by reacting the dibutyltin(IV) dichloride, diphenyltin(IV) dichloride and triphenyltin(IV) chloride respectively via the dibutyltin(IV) oxide, diphenyltin(IV) dihydroxide and triphenyltin(IV) hydroxide with benzoate acid and its derivative. The targeted compounds have been tested with anticancer activity against leukemia cancer cell, L-1210. The compounds synthesized were well characterized by ^{1}H and ^{13}C-NMR, IR and UV-Vis spectroscopies as well as based on the microanalytical data. The results showed that triphenyltin(IV) benzoate and its derivative prepared exhibit higher anticancer activity than those of dibutyltin(IV) and diphenyltin(IV) analogous.

Keywords: *anticancer; IC_{50}; leukemia cancer cell; organotin(IV) benzoates*

ABSTRAK

Telah dibuat seri senyawa dibutil-, difenil- dan trifeniltimah(IV) dengan asam benzoat dan turunannya. Senyawa hasil diperoleh dengan mereaksikan berturut-turut dibutiltimah(IV) diklorida, difeniltimah(IV) diklorida dan trifeniltimah(IV) klorida melalui senyawa antara dibutiltimah(IV) oksida, difeniltimah(IV) dihidroksida dan trifeniltimah(IV) hidroksida dengan asam benzoat dan turunannya. Senyawa-senyawa yang dihasilkan kemudian diuji aktifitas antikankernya terhadap sel kanker leukemia L-1210. Senyawa-senyawa hasil sintesis dikarakterisasi dengan spektroskopi NMR ^{1}H dan ^{13}C, IR dan UV sinar tampak dan juga berdasarkan data mikroanalisis. Hasil penelitian menunjukkan bahwa senyawa trifeniltimah(IV) benzoat dan turunannya memberikan hambatan tertinggi pada uji antikanker dibandingkan senyawa sejenis dari dibutiltimah(IV) dan difeniltimah(IV).

Kata Kunci: *antikanker; IC_{50}; sel kanker leukemia; organotimah(IV) benzoat*

INTRODUCTION

The structural chemistry of organotin carboxylates has attracted much attention due to their significant cytotoxic effect and relatively high antitumor activity [1-2]. The new development of these compound may lead for the development of new antitumor drugs, which can display another spectrum of antitumor activity [1,3]. The organotin(IV) compounds are known to display strong biological activity. Their compounds are normally exhibiting high toxicity, even at very low concentration. Their biological activities are fundamentally determined by the number and the nature of organic groups bound to the central Sn atom [4]. The nature of the anionic groups seems acting only as a secondary factor.

The current investigations on the coordinating properties of carboxylates toward organotin compounds have led to the isolation of some new organotin(IV) carboxylates and carboxylate derivatives which have shown some interesting biological activities such as antimicrobial [5-6], antitumor and anticancer [1,3,7-9], and antifungal activity [6,10-12]. The investigation of organotin(IV) as possible antitumor compound has been and is still attracting much attention [1,3,7-9].

In the present work, we reported application, comparative and in vitro activity study of some dibutyl-, diphenyl- and triphenyltin(IV) benzoate and its derivatives against leukemia cancer cell, L-1210.

EXPERIMENTAL SECTION

Materials

All reagents used were AR grade. Dibutyltin(IV) dichlorides ([(n-$C_4H_9)_2Cl_2$]), diphenyltin(IV) dichloride ([$(C_6H_5)_2Cl_2$]), triphenyltin(IV) chloride ([$(C_6H_5)_3Cl$]), benzoic acid, 2-hydroxybenzoic acid, sodium hydroxide (NaOH), methanol (CH_3OH) were either Sigma or JT Baker products, and were used without further purification. The leukemia cancer cells, L-1210, were obtained from Center for Application of Isotopes and

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Instrumentation

Elemental analysis (CHNS) was performed on Fision EA 1108 series elemental analyzer. IR spectra in the range of 4000-400 cm^{-1} were recorded on a Bruker VERTEX 70 FT-IR spectrophotometer with KBr discs. The UV spectra were recorded in the UV region and were measured using a UV-Shimadzu UV-245 Spectrophotometer. Measurements were performed in 1 mL quartz-cells. Solutions were prepared using methanol as the solvent with concentration of 1.0 x 10^{-4}M. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 600 MHz NMR (600 MHz for ^{1}H and 150 MHz for ^{13}C). All experiments were run in DMSO-D_6 at 298 K. The number of runs used for ^{1}H experiments were 32 with reference at DMSO signal at 2.5 ppm, while the ^{13}C were 1000-4000 scans with the reference DMSO signal at 39.5 ppm.

Procedure

Preparation of organotin(IV) carboxylates

The organotin(IV) carboxylates used in this work and similar compounds with variety of benzoic derivatives as the ligands were prepared based on the procedure previously reported [9,11-12], and was adapted from the work by Szorcsik et al. [13]. An example procedure in the preparation of dibutyltin(IV) dibenzoate is as follows:

To 3.0383 g (0.01 mol) [(*n*-$C_4H_9)_2SnCl_2$] in 50 mL methanol was added 0.8 g (0.02 mol) NaOH. The reaction mixtures were stirred for about 45 min. Compound **2** was precipitated out as white solid, filtered off and dried *in vacuo* till they are ready for analysis and further reaction. The average yield was 2.3508 g (95%).

To 0.37338 g (1.5 mmol) compound **2** in 50 mL of methanol was added with 2 mole equivalents of benzoic acid and was refluxed for 4 h at 60–70 °C. After removal of the solvent by rotary evaporator, the produced compounds [(*n*-$C_4H_9)_2Sn(OOCR)_2$] were dried *in vacuo* until they are ready for analysis and further use for biological test. The average yields were more than ~90%.

A similar procedure was also adapted in the preparation of diphenyltin(IV) and triphenyltin(IV) derivatives, [$(C_6H_5)_2Sn(OOCR)_2$] and [$(C_6H_5)_3Sn(OOCR)$], respectively. For triphenyltin(IV) only one mole equivalent of the carboxylic acid was added.

Bioassay anticancer activity test against leukemia cancer cell, L-1210

The *in vitro* anticancer activity test against leukemia cancer cell, L-1210 was performed based on the known procedure [9]. 1 mL of the cancer cells were added into each hole of *multiwell plate tissue culture* containing 2 x 10^6 cell/mL followed by the addition of 10 μL of solution containing the compounds tested in methanol (if the sample was not soluble enough in the solvent used, before it is being added, the ultrasonic mixing was done first to homogenize the sample). The sample concentration variations used were 1, 2, 4, 6, 8, 16, 32 μg/mL and three replicates per concentration were performed. As the negative control, the cell was treated with a solution containing 10 μL of solvent was used as comparison. The cell was then incubated for 48 h in 5% CO_2 incubator at 37 °C. After being incubated, the sum of cell was counted in microscope using *haemecytometer Fuch Rosental* (0.200 mm x 0.0625 mm^2).

The percentage of inhibition was calculated using the following formula in Eq. (1):

$$\% \text{ inhibiton} = \left(1 - \frac{A}{B}\right) \times 100 \quad (1)$$

where A is the number of lives cell in medium tested; and B is the number of lives cell in control/blank.

Calculation of IC_{50}

The IC_{50} was calculated according to the adaption of Reed and Muench method which is an arithmetic method [14] and as following:

The data of all inhibition percentages were plotted into probit table to obtain each probit value of each analysis. The graph between log of concentration (x) and probity value (y) was then created to obtain a linier regression, y = a + bx. By inputting the probit value (y) = 5 (the probit of 50% value), then the value of x (log of concentration) was obtained. The IC_{50} value was then obtained by taking the anti log of x.

RESULT AND DISCUSSION

Preparation and characterization of organotin(IV) carboxylate compounds

The preparation of dibutyltin(IV) dicarboxylates, [(*n*-$C_4H_9)_2Sn(OOCR)_2$] (**3, 4**), diphenyltin(IV) dicarboxylates [$(C_6H_5)_2Sn(OOCR)_2$] (**7, 8**) and triphenyltin(IV) carboxylates, [$(C_6H_5)_3Sn(OOCR)$] (**11, 12**), were successfully done from their chlorides [(*n*-$C_4H_9)_2SnCl_2$] (**1**), [$(C_6H_5)_2SnCl_2$] (**5**) and [$(C_6H_5)_3SnCl$] (**9**), respectively. To maximize the product

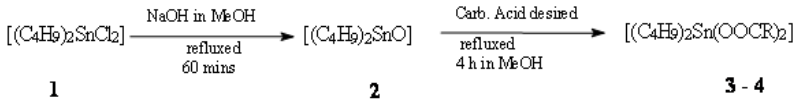

Fig 1. The scheme of preparative route of the organotin(IV) dicarboxylate

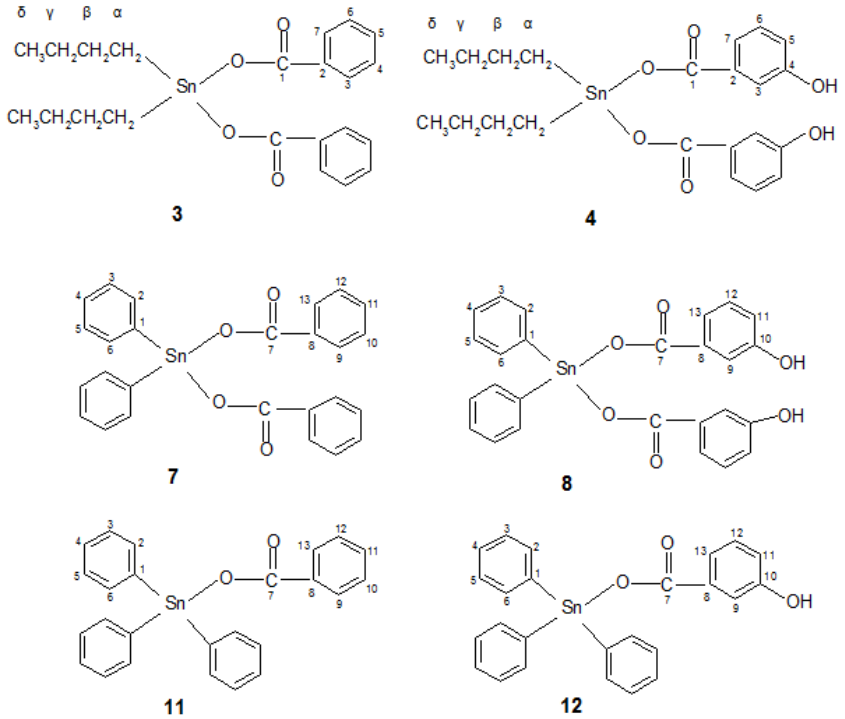

Fig 2. The proposed structure of the compounds synthesized and the suggested numbering of carbons in each compound

obtained, the reactions in all cases were done *via* [(*n*-$C_4H_9)_2SnO$] (**2**), [$(C_6H_5)_2Sn(OH)_2$] (**6**) and [$(C_6H_5)_3SnOH$] (**10**) respectively similar to those previously reported [9,11-12]. The reaction occurred in each step for dibutyltin(IV) dicarboxylates, for example, is shown in Scheme 1. The microanalytical data of all compounds prepared are very good and all values obtained are close to the calculated values as shown in Table 1.

The characterizations of the targeted products synthesized were confirmed by some spectroscopy techniques. The ^{1}H and ^{13}C chemical shifts of the compounds prepared are shown Table 2. A number of signals in the spectra recorded have been characterized carefully. The chemical shift (δ) of butyl protons attached to the tin metal appeared in the range of 0.93 ppm for Hδ up to 1.4-1.6 ppm Hα and Hβ, and the carbons of butyl ligands are observed at position comparable with other similar compounds reported by other [15]. The chemical shift of phenyl protons attached to tin metal appeared in the range of 7.6–7.36 ppm, while the carbon of carboxyl group of all compounds as expected appeared in the region of 174 ppm [15, 16]. The carbon atoms of the phenyl ligand as also expected appeared in δ of 130–126 ppm, while the carbons in the benzoate derivatives appeared in δ range of 139–129 ppm close to the reported values of similar compounds [16].

The important FT-IR data and their assignments are presented in Table 3. The characteristic band of the starting materials (**1, 5, 9**) is the appearance of strong stretching band of Sn-Cl bond at 390–310 cm^{-1}. As an example in the spectrum of **1**, this bond appeared at frequency of 334.2 cm^{-1}. The other characteristic bands of this compound appear as stretching band from butyl ligands at 1069 cm^{-1}, and bending vibration of C-H aliphatic stretch of the butyl at frequency of 2956–2865 cm^{-1}.

When compound **1** is converted to compound **2**, the main stretching band of Sn–Cl disappeared and a new strong band at frequency of 417.4 cm^{-1} appeared as one of the main stretching band. This band is characteristic for Sn–O bond in compound [(*n*-$C_4H_9)_2SnO$] (**2**). The stretching band due to the butyls and their bending vibrations are still appearing as expected although the frequencies have little bit shifted. The formation of dibutyltin (IV) dicarboxylate compounds, [(*n*-$C_4H_9)_2Sn(RCOO)_2$], (**3, 4**) is confirmed

Table 1. The microanalytical data of the organotin(IV) compounds synthesized

Compound	Elemental analysis found (calculated)	
	C	H
[(*n*-C_4H_9)$_2$$SnCl_2$] (**1**)	31.4 (31.6)	6.2 (6.0)
[(*n*-C_4H_9)$_2$SnO] (**2**)	38.6 (38.6)	7.2 (7.3)
[(*n*-C_4H_9)$_2$Sn(C_6H_5COO)$_2$] (**3**)	55.1 (55.6)	5.9 (6.0)
[(*n*-C_4H_9)$_2$Sn(*o*-C_6H_4(OH)COO)$_2$] (**4**)	52.3 (52.1)	5.7 (5.6)
[(C_6H_5)$_2$$SnCl_2$] (**5**)	41.6 (41.9)	2.8 (2.9)
[(C_6H_5)$_2$Sn(OH)$_2$] (**6**)	46.5 (46.9)	3.8 (3.9)
[(C_6H_5)$_2$Sn(C_6H_5COO)$_2$] (**7**)	60.4 (60.6)	3.6 (3.7)
[(C_6H_5)$_2$Sn(*o*-C_6H_4(OH)COO)$_2$] (**8**)	56.3 (57.0)	3.6 (3.7)
[(C_6H_5)$_3$SnCl] (**9**)	55.8 (56.1)	4.0 (3.9)
[(C_6H_5)$_3$Sn(OH)] (**10**)	58.4 (58.9)	4.3 (4.4)
[(C_6H_5)$_3$Sn(C_6H_5COO)] (**11**)	63.9 (63.7)	4.4 (4.3)
[(C_6H_5)$_3$Sn(*o*-C_6H_4(OH)COO)] (**12**)	60.7 (61.1)	4.2 (4.1)

Table 2. ^{1}H and ^{13}C spectra of the compounds synthesized

Compounds	**H** in butyl or phenyl (ppm)	**H** in benzoate (ppm)	**C** in butyl, phenyl and benzoate (ppm)
[(*n*-C_4H_9)$_2$Sn($OOCC_6H_5$)$_2$] (**3**)	Hα & Hβ:1.4-1.6 (m); Hγ: 1.29 (m); Hδ: 0.93 (t)	7.35-7.85 (m)	Cα: 21.3; Cβ: 26.6; Cγ: 25.9; Cδ: 14.2; C1: 174.2; C2: 139.3; C3 & C7: 129.7; C4 & C6: 128.6; C5: 125.1
[(*n*-C_4H_9)$_2$Sn(*o*-C_6H_4(OH)COO)$_2$] (**4**)	Hα & Hβ:1.4-1.6 (m); Hγ: 1.29 (m); Hδ: 0.93 (t)	7.35-7.89 (m)	Cα: 21.3; Cβ: 26.7; Cγ: 25.9; Cδ: 14.3; C1: 174.2; C2: 139.3; C3: 130.1; C4: 164.6; C5: 129.5; C6: 128.9; C7: 129.9
[(C_6H_5)$_2$Sn($OOCC_6H_5$)$_2$] (**7**)	H2 & H6 7.59 (d, 4H); H3 & H5 7.48 (t, 4H); H4: 7.35 (t, 2H)	7.81-7.94 (m)	C1-6 (phen): 131.7-126.9; C7: 174.7; C8: 139.5; C9 & C13: 130.2; C10 & C12: 129.1; C11: 128.5
[(C_6H_5)$_2$Sn(*o*-C_6H_4(OH)COO)$_2$] (**8**)	H2 & H6 7.58 (d, 4H); H3 & H5 7.48 (t, 4H); H4 7.36 (t, 2H)	7.89-7.99 (m)	C1-6 (phen): 131.7-126.9; C7: 174.9; C8: 139.8; C9: 130.4; C10: 164.9; C11: 129.7; C12: 129.0; C13: 130.2
[(C_6H_5)$_3$Sn($OOCC_6H_5$)] (**11**)	H2 & H6 7.57 (d, 6H); H3 & H5 7.45 (t, 6H); H4: 7.31 (t, 3H)	7.81-7.89 (d)	C1-6 (phen): 131.1-126.2; C7: 174.4; C8: 139.3; C9 & C13: 130.1; C10 & C12: 128.7; C11: 128.2
[(C_6H_5)$_3$Sn(*o*-C_6H_4(OH)COO)] (**12**)	H2 & H6 7.57 (d, 6H); H3 & H5 7.45 (t, 6H); H4: 7.31 (t, 3H)	7.86-7.92 (d)	C1-6 (phen): 131.1-126.2; C7: 174.8; C8: 139.6; C9: 130.1; C10: 164.2; C11: 129.3; C12: 128.7; C13: 129.8

by the strong asymmetric stretching bands of the carboxylates which occurred at *ca.* 1400 cm^{-1} and the symmetric stretch at *ca.* 1600 cm^{-1}, confirming the success of the substitution reaction [9,11-12].

The UV-Vis spectroscopy analyses have also been taken for all the compounds used. The λ_{max} of all the compounds is summarized in Table 4. From the data obtained (Table 3), it is clear that there was a shifting change in the λ_{max} for each compound in any steps of the reaction. For example, the compound **1** has λ_{max} of 210.7 nm, while compound **2** has λ_{max} of 202.9 nm. This information gave an indication that there was a shift to a shorter λ_{max} value when the conversion of compound **1** to **2** takes place. The wave-length shift to a shorter λ_{max} could occur due to either the solvent used or the effect of an auxochrome. However in this study, as the solvent used for all measurements was the same (methanol), these changes must be due to the auxochrome effect. In the case of compound **1** and **2**, there is an oxide group which has electron drawing effect bigger in compound **2** than that of chloride group in **1**. As a result, the electron transition in **2** is hard to occur. Thus, the measured λ_{max} was getting shorter in compound **2** than in compound **1** [17]. Similar results are also observed for other changes as can be seen from Table 3. For example, in compound **3**, the electron drawing effect of *o*-C_6H_4(OH)COOH is less than

Table 3. The characteristic and important IR bands of the organotin(IV) compounds (cm^{-1}) synthesized

Compound	**3**	**4**	**7**	**8**	**11**	**12**	References
Sn-O	434.5	435.7	594.7	591.6	765.59	755.41	800-400
Sn-O-C	1029.9	1028.1	1243.4	1290.1	1243.36	1298.7	1050-900
Sn-Bu	674.8	678.3	-	-	-	-	740-660
CO_2 asym	1419.6	1418.2	1532.9	1596.8	1558.8	1562.3	1600-1400
CO_2 sym	1558.7	1560.7	1660.8	1690.2	1631.36	1698.74	1700-1550
C-H aliphatic	2955–2862	2955–2866	-	-	-	-	2960–2850
Phenyl	-	-	1467.6; 751.3	1490.8; 725.2	1428.7; 729.64	1430.2; 729.53	1450, 730

Table 4. The λ_{max} of the UV-Vis spectra of the organotin(IV) compounds

Compound	λ_{max} (nm)
[(*n*-C_4H_9)$_2$SnCl] (**1**)	210.7
[(*n*-C_4H_9)$_2$SnO] (**2**)	202.9
[(*n*-C_4H_9)$_2$Sn(C_6H_5COO)$_2$] (**3**)	302.3
[(*n*-C_4H_9)$_2$Sn(*o*-C_6H_4(OH)COO)$_2$] (**4**)	307.8
[(C_6H_5)$_2$Sn(C_6H_5COO)$_2$] (**7**)	283.6
[(C_6H_5)$_2$Sn(*o*-C_6H_4(OH)COO)$_2$] (**8**)	287.7
[(C_6H_5)$_3$Sn(C_6H_5COO)] (**11**)	298.9
[(C_6H_5)$_3$Sn(*o*-C_6H_4(OH)COO)] (**12**)	302.3

Table 5. IC_{50} values of all compounds tested

Compounds	IC_{50} (µg/mL)
[(*n*-C_4H_9)$_2$Sn(C_6H_5COO)$_2$] (**3**)	19.6
[(*n*-C_4H_9)$_2$Sn(*o*-C_6H_4(OH)COO)$_2$] (**4**)	24.4
[(C_6H_5)$_2$Sn(C_6H_5COO)$_2$] (**7**)	9.2
[(C_6H_5)$_2$Sn(*o*-C_6H_4(OH)COO)$_2$] (**8**)	10.3
[(C_6H_5)$_3$Sn(C_6H_5COO)] (**11**)	5.3
[(C_6H_5)$_3$Sn(*o*-C_6H_4(OH)COO)] (**12**)	3.8

chloride in **1**, so the electron transition in this molecule will be easier (the energy required is less), thus producing longer λ_{max}, 307.8 nm.

The antifungal activity of diorganotin(IV) and triorganotin(IV) compounds are well known, and many of their compounds have been synthesized for that purpose [6,10-12]. Some of the organotin(IV) compounds prepared have also been tested for antitumor activity and showed quite high antitumor activity [1,3,7-9].

In our previous study on the antifungal activity of the compounds reported here [11-12], it has been shown that optimal activity of the antifungal has been associated with the number of carbon atoms present in the organotin(IV) used [18], where in general, the derivative of triphenyltin(IV) carboxylate which contain 18 carbon atoms has smallest minimum inhibition concentration values in the series [11-12]. In this study interestingly the same phenomena was also observed.

Based on the data in Table 5, the derivatives of triphenyltin(IV) compounds has smaller IC_{50} value in the series, and the diphenyltin(IV) compound has smaller IC_{50} value than those of dibutyltin(IV) compounds. Thus the number of carbon atoms present has effect on the anticancer activity of the organotin(IV) tested. Besides that, the organotin(IV) carboxylate compounds synthesized have smaller IC_{50} values compare to those of starting materials and intermediate products. In this respect, our results are consistent with a well-known fact that many biologically active compounds become more active upon complexation than in their uncomplexed forms [19]. According to Crowe [20] the actual biological activity of diorganotin compounds of the type RR'SnXY (R and R' = alkyl or aryl; X and Y = anions) is determined solely by the $RR'Sn^{2+}$ moiety. Consequently the group X and Y would only influence the delivery of the active $RR'Sn^{2+}$ ion to the cell, although it is not clear enough how is their mechanism of action.

CONCLUSION

It is quite clear from the discussion above that some promising organotin(IV) carboxylate compounds were successfully prepared and exhibited very high *in vitro* anticancer activity. The study of other organotin(IV) series which might have higher and better anticancer activity is still in progress. The *in vivo* testing of the compounds synthesized against human cancer will also be undertaken to evaluate their possible use as an anticancer metal base-drug in the future.

ACKNOWLEDGEMENT

The authors would like to thank to The Directorate of Research and Services, Directorate General of Higher Education, The Ministry of Cultural and Education of Republic of Indonesia that provide fund for this project to be undertaken through Hibah Kompetensi Batch 2 Research Grant Scheme 2009 and 2011 with contract numbers of 254/SP2H/PP/DP2M/V/2009 and 364/SP2H/PL/Dit. Litabmas/IV/2011, respectively. Thanks also go to Prof. Bohari M. Yamin, Universiti Kebangsaan Malaysia for helping in doing microanalysis and Dr. Huy Hoang of Institute of Molecular Biosciences (IMB) University of Queensland, Brisbane, Australia for NMR experimentation.

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