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Structure–Activity Relationship Studies of Ethyl 2-[(3-Methyl-2,5-dioxo(3-pyrrolinyl))amino]-4-(trifluoromethyl)pyrimidine-5-carboxylate: An Inhibitor of AP-1 and NF-κB Mediated Gene Expression

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Abstract—Several analogues of ethyl 2-[(3-methyl-2,5-dioxo(3-pyrrolinyl))amino]-4-(trifluoromethyl)pyrimidine-5-carboxylate (**1**) were synthesized and tested as inhibitors of AP-1 and NF-κB mediated transcriptional activation in Jurkat T cells. From our SAR work, ethyl 2-[(3-methyl-2,5-dioxo(3-pyrrolinyl))-*N*-methylamino]-4-(trifluoromethyl)-pyrimidine-5-carboxylate was identified as a novel and potent inhibitor.

T-lymphocytes (T-cell) orchestrate both the initiation and propagation of various immune responses through the secretion of protein mediators termed cytokines.[1] These cytokines play a significant role in a number of inflammatory diseases such as asthma, psoriasis, rheumatoid arthritis, and transplant rejection.[2] Several studies have shown that T-cell driven immune responses appear to overreact in these disease states.[3,4] In activated T cells, transcription factors such as the activator protein-1 (AP-1), regulate IL-2 and matrix metalloproteinases production, while the nuclear factor-κB (NF-κB), is essential for the transcriptional regulation of the proinflammatory cytokines IL-1, IL-6, IL-8, and TNF-α.[5] Based on these findings, it appears that inhibition of AP-1 and NF-κB transcriptional activation in T cells may represent an attractive target in the development of novel antiinflammatory drugs.[6]

Very few compounds are known to inhibit both the AP-1 and NF-κB mediated transcriptional activation.[7] A pyrrole analogue, PNU156804, was shown to block the activation of both AP-1 and NF-κB transcription factors.[8] Macrolide antibiotic such as Erythromycin at therapeutic concentrations inhibited AP-1 and NF-κB mediated transcriptional activation.[9] We screened our diversity library using automated high-throughput assays with stably transfected human Jurkat T-cells and identified a novel compound (**1**) that inhibited both AP-1 and NF-κB mediated transcriptional activation ($IC_{50} = 2$ μM). Compound **1** did not block basal transcription driven by the β-actin promoter. In addition, **1** had an inhibitory effect on the production of IL-2 and IL-8 levels in α stimulated Jurkat T-cells. Our goal was to improve the potency of **1** by exploring different substituents around the 2, 4, and 5-positions of the pyrimidine ring. This paper describes the synthesis and structure-activity relationship of this series of novel compounds (Fig. 1).

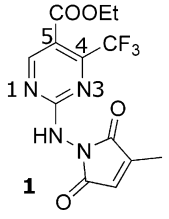

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Figure 1.

Scheme 1 shows the synthesis of various analogues of **1** that contain different groups at the 4- and 5-position of

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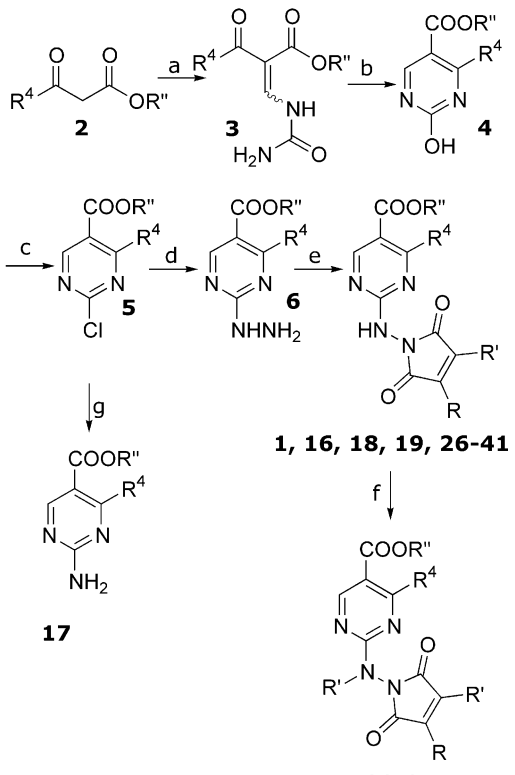
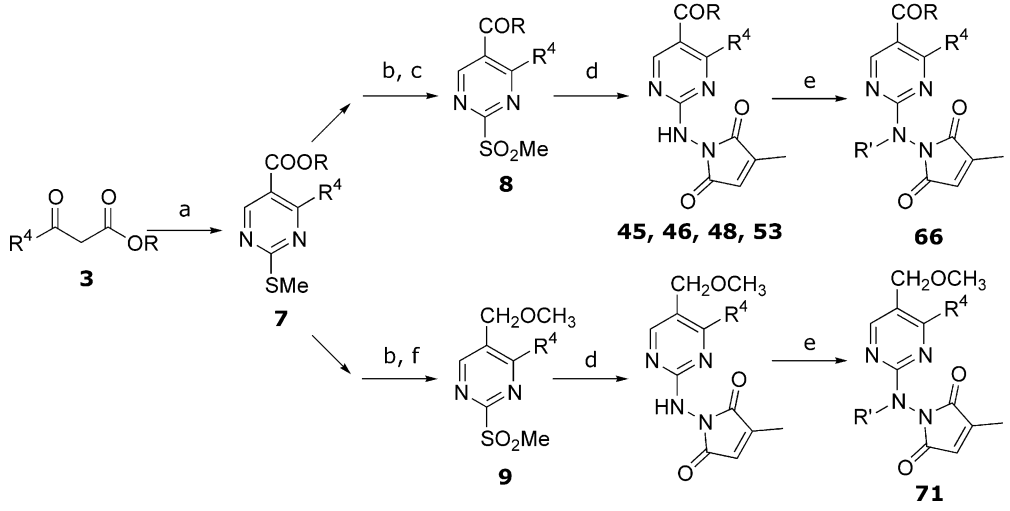


Scheme 1. (a) Urea, $CH(OEt)_3$, reflux, overnight; (b) NaOEt, EtOH, rt, overnight; (c) $POCl_3$, reflux, N_2, 3 h; (d) hydrazine, THF, rt, 5 min; (e) citraconic anhydride, chloroform, reflux, 3 h; (f) R^1Br, NaH, THF, rt, overnight, (for R^1 = alkyl) or RCOCl, pyridine, rt, overnight (for R^1 = RCO) or RSO_2Cl, pyridine, rt, overnight, (for R^1 = RSO_2) or RNCO, THF, rt, overnight, (for R^1 = RNHCO) or ROCOCl, pyridine, rt, overnight (for R' = ROCO); (g) NH_3, THF, 0 °C to rt, 6 h.

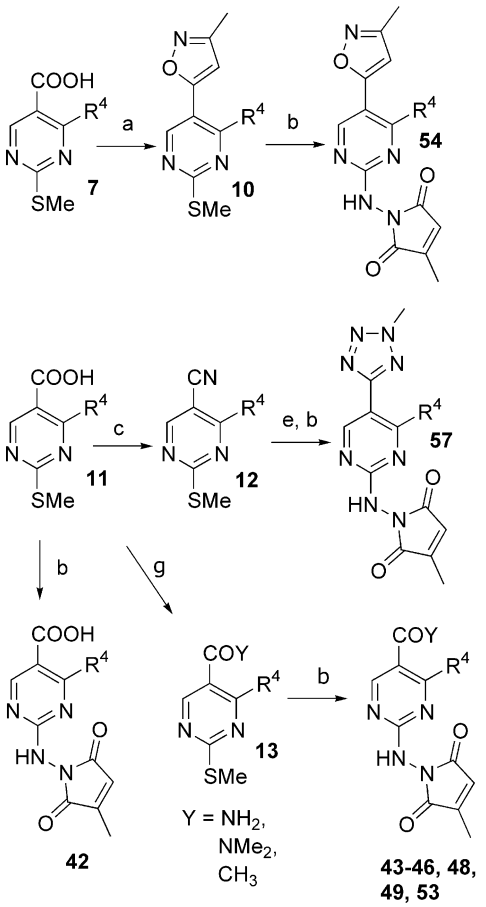
the pyrimidine ring. An appropriately substituted β-keto ester was heated at reflux with urea and triethyl orthoformate to give **3**. A base promoted cyclization of the intermediate **3** resulted in a pyrimidine ring **4**.[10] The hydroxy group in **4** was converted to a chloro by treating with phosphorus oxychloride.[11] The chlorine was first displaced with hydrazine and then converted to a 3-methyl-2, 5-dioxo(3-pyrrolinyl) or citraconamido group by heating at reflux with citraconic anhydride. The substituents on the secondary amine at the 2-position was introduced either by treating **5** with an alkyl halide and a base or an acid chloride or an isocyanate or a sulphonyl chloride or a chloroformate. The chloro intermediate **5** was treated with ammonia to give a 2-amino analogue **17**.

The synthesis of 5-substituted ketones and ethers is shown in Scheme 2. An appropriately substituted β-ketoester was cyclized with *S*-methylisothiourea under basic conditions to give pyrimidine intermediate **7**. The ester group was hydrolyzed, converted to acid chloride using oxalyl chloride and then treated with a Grignard reagent to give a keto analogue. The *S*-methyl group was oxidized to a sulphone, **8**. Treatment of the sulphone with hydrazine followed by citraconic anhydride resulted in compounds **45**, **46**, **48**, and **53**. An alkyl group was introduced on the secondary amine by treatment with an appropriate alkyl halide and sodium hydride in THF to give compound **66**. The synthesis of ether analogue (**71**) started with the ester **7**, which was hydrolyzed and reduced to an alcohol using mixed anhydride and sodium borohydride. The intermediate alcohol was methylated using iodomethane. The *S*-Me group was then converted to a citraconamido group as described earlier to give **71**.

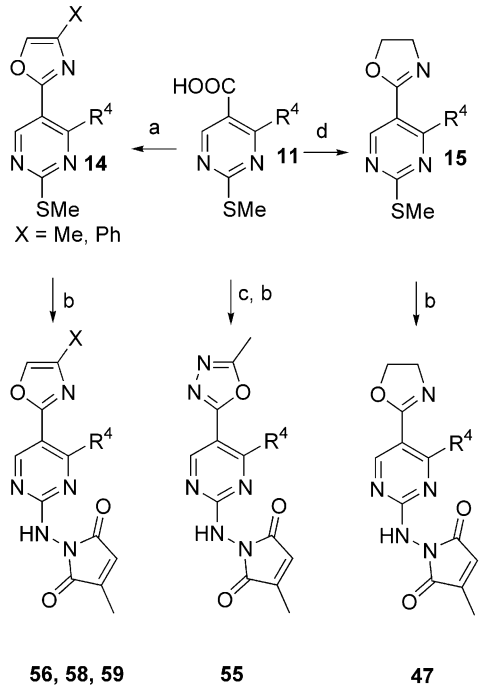
Scheme 3 illustrates the introduction of various groups such as amides, ethers, and ester bioisosteres at the 5-position of the pyrimidine ring. A carboxylic acid in compound **7** was treated with acetone oxime and *n*-butylithium, followed by acid to give an isoxazole, **10**. The *S*-Me group in **10** was converted to a citraconamido group, as described above, to give compound, **54**. The carboxylic acid in **11** was converted to a cyano group by treating **11** with oxalyl chloride, followed by



Scheme 2. (a) *S*-methylisothiourea, NaOEt, EtOH, reflux, overnight; (b) NaOH, THF-methanol-water, rt, overnight; (c) (i) $(COCl)_2$, DCM, rt, 1 h; (ii) RMgBr, THF, −78 °C, 2 h; (iii) *m*-CPBA; DCM, rt, overnight; (d) (i) Hydrazine, THF, rt, 5 min; (ii) citraconic anhydride, chloroform, reflux, overnight; (e) R'Br, NaH, THF, rt, overnight (R' = alkyl); (f) (i) Isobutylchloroformate; *N*-methyl morpholine (NMM), sodium borohydride, water, rt, 1 h, (ii) methyl iodide, silver (II) oxide, acetonitrile, rt, overnight, (iii) *m*-CPBA; DCM; rt; overnight.



Scheme 3. (a) (i) Acetone oxime, *n*-BuLi, THF; (ii) H_2SO_4, THF, (b) (i) *m*-CPBA, DCM, rt, overnight, (ii) Hydrazine, THF, rt, 5 min; (iii) citraconic anhydride, chloroform, reflux, overnight; (c) (i) $(COCl)_2$, DCM, rt, 2 h, (ii) NH_3 (gas), THF, rt, overnight, (iii) $SOCl_2$; DMF; (e) (i) NaN_3; NH_4Cl, DMF, (ii) MeI, K_2CO_3; (g) (i) $COCl_2$, DCM, 1 h, rt, (ii) YH, THF, 1 h, rt.



Scheme 4. (a) (i) $ClCH_2COCH_3$, (ii) acetamide, BF_3–Et_2O, xylenes; (b) (i) *m*-CPBA, (ii) hydrazine, THF, rt, 5 min; (iii) citraconic anhydride, chloroform, reflux, overnight; (c) (i) $CH_3CONHNH_2$, $POCl_3$, reflux, overnight; (d) (i) $(COCl)_2$, (ii) $NH_2CH_2CH_2OH$; (iii) $SOCl_2$, EtOAc–$CHCl_3$.

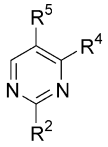
ammonia and then finally with thionyl chloride. The *N*-methylated tetrazole was obtained by treating the cyano analogue (**12**) with sodium azide and ammonium chloride followed by methylation using iodomethane. The citraconamido group was introduced at the 2-position of compound **12**, as described earlier, to give **57**. Similarly, the carboxylic acid analogue **42** was synthesized from **11** as shown. The carboxylic acid of **11** was also converted to an ester or amide via an acid chloride to give intermediate **13**. A citraconamido group was introduced at the 2-position, as described above, to give **43**–**46**, **48**, **49**, and **53**.

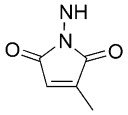
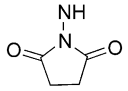
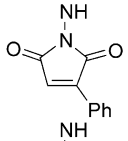
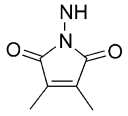
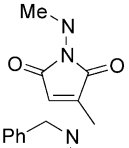
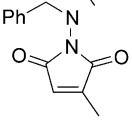
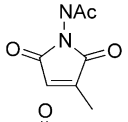
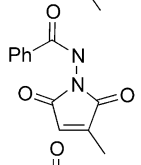
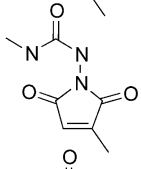
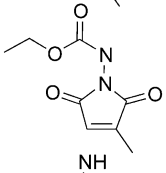
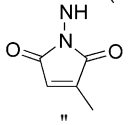
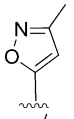
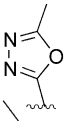
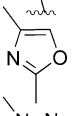
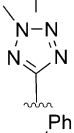
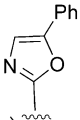
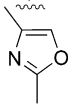
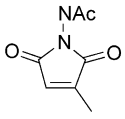
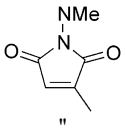
The synthesis of additional ester bioisosteres is illustrated in Scheme 4. Treatment of the carboxylic acid analogue **11** with chloroacetone, followed by acetamide in BF_3-etherate provided the methyl oxazole **14**. The intermediate **14** was converted to **56**, **58**, and **59** as described previously.[12] Oxadiazole analogues (**55**) were synthesized by treating **11** with *N*-acetylhydrazide and phosphorus oxychloride, followed by hydrazine and then citraconic anhydride.[13] The carboxylic acid in **11** was converted to a dihydro oxazole analogue (**15**) by treating with oxalyl chloride, followed by aminoethanol. The intermediate **15** was converted to the citraconamide analogue **47** as described before.[14]

The analogues synthesized as part of this study were evaluated in Jurkat T-cells stably transfected with promoter-reporter gene constructs driven by either an AP-1 binding site or a NF-κB binding site.[15,16] All the compounds were tested in both assays. The IC_{50} values are shown in Table 1. Since all the compounds had similar IC_{50} values in both AP-1 and NF-κB assays, average IC_{50} values in both assays are shown in Table 1.

Compound **1** was active in both the cell-based assays ($IC_{50} = 2$ μM). Initial SAR studies were focused on modifying 3-methyl-2,5-dioxo(3-pyrrolinyl)amino or citraconamido group. The removal of a methyl group (**16**), or the introduction of a phenyl group in the place of methyl (**18**) or the introduction of a 2nd methyl group on the citracanamido ring (**19**) resulted in very little change in activity. However, when the citraconamido ring was removed, compound **17** had much weaker potency. Next, we examined the importance of NH at the 2-position. The hydrogen was replaced with alkyl groups (**20** and **21**), substituted carbonyl groups (**22** and **23**), a urea group (**24**), and a carbamate group (**25**). All these compounds showed 2- to 6-fold improvement in potency. The importance of a trifluoromethyl group at the 4-position was also examined. Removal of the trifluoromethyl group (**26**) resulted in a 6-fold loss in potency. Substitution of the trifluoromethyl with a methyl (**27**) resulted in a compound with comparable potency. However, groups such as ethyl- (**28**) and pentafluoroethyl- (**29**) substituted compounds resulted in 5- to 10-fold increase in potency. Other bulky groups such as phenyl (**30**) and benzyl (**31**) did not improve the potency, and methoxymethyl (**32**)

Table 1. Inhibition of AP-1 and NF-κB-mediated transcriptional activation in Jurkat cells



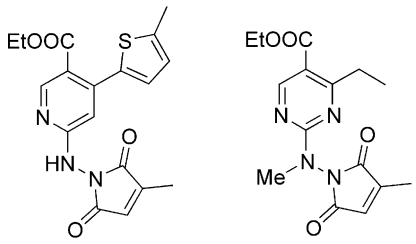
No.	R^2	R^4	R^5	IC_{50}, μM
1		CF_3	CO_2Et	2
16		CF_3	CO_2Et	1.6
17	NH_2	CF_3	CO_2Et	30
18		CF_3	CO_2Et	3.7
19		CF_3	CO_2Et	3.9
20		CF_3	CO_2Et	0.3
21		CF_3	CO_2Et	0.45
22		CF_3	CO_2Et	0.41
23		CF_3	CO_2Et	0.83
24		CF_3	CO_2Et	0.59
25		CF_3	CO_2Et	0.38
26		H	CO_2Et	13
27	"	CH_3	CO_2Et	1.9
28	"	CH_2CH_3	CO_2Et	0.4
29	"	CF_2CF_3	CO_2Et	0.2
30	"	Ph	CO_2Et	1.2
31	"	CH_2Ph	CO_2Et	3.8
32	"	CH_2OCH_3	CO_2Et	6.0
33	"	2-Furanyl	CO_2Et	1
34	"	2-Thienyl	CO_2Et	0.14
35	"	3-Thienyl	CO_2Et	0.20
36	"	2-(5-Methylthienyl)	CO_2Et	0.045
37	"	2-(5-Chlorothienyl)	CO_2Et	0.52
38	"	2-Benzo[b]thienyl	CO_2Et	0.8
39	"	2-Thiazolyl	CO_2Et	2.2
40	"	Cyclopropyl	CO_2Et	1.5
41	"	CF_3	CO_2-tBu	0.21
42	"	CF_3	CO_2H	30
43	"	CF_3	$CONH_2$	30
44	"	CF_3	$CONMe_2$	30
45	"	CF_3	COMe	4.4
46	"	CF_3	COPh	0.098
47	"	CF_3		6.4
48	"	CH_2CH_3	COPh	0.65
49	"	CH_2CH_3	$COCH_2CH_2CH_3$	0.45
50	"	CH_2CH_3	Cyclopropyl ester	0.12
51	"	CH_2CH_3	CH_2OH	4.9
52	"	CH_2CH_3	CN	1.1
53	"	CH_3	$COCH_3$	7.7
54	"	CH_2CH_3		10
55	"	CH_2CH_3		10
56	"	CH_2CH_3		0.83
57	"	CH_2CH_3		10
58	"	CH_2CH_3		2.8
59	"	2-Thienyl		0.76
60	"	2-Thienyl	CO_2-*t*Bu	0.05
61		$CH_2CH_2CH_3$	CO_2Et	0.43
62		CF_2CF_3	CO_2Et	0.45
63	"	CH_2CH_3	CO_2Et	0.035
64	"	2-Benzo[*b*]thienyl	CO_2Et	0.13
65	"	2-Thiazolyl	CO_2Et	1.7
66	"	CH_2CH_3	COPh	0.41
67	"	CF_2CF_3	CO_2Et	0.35
68	"	2-Thienyl	CO_2Et	0.094
69	"	3-Thienyl	CO_2Et	0.12
70	"	2-(5-Methylthienyl)	CO_2Et	0.35
71	"	CH_2CH_3	CH_2OCH_3	0.63

resulted in decreased potency. The introduction of a 2-thienyl group (**34**) resulted in more than 10-fold improvement in potency. The introduction of 2-(5-methylthienyl) group (**36**) resulted in a compound with more than 40-fold improvement in potency. The significance of the ethyl carboxylate at the 5-position of the ring was also examined. The introduction of a bulky alkyl group such as *t*-butyl group (**41**) in the place of ethyl resulted in 10-fold increase in potency. However, groups such as a carboxylic acid (**42**), carboxamide (**43**), and *N*,*N*-dimethyl carboxamide (**44**) all resulted in the loss of potency. While a methyl ketone (**45**) at the 5-position resulted in small improvement in potency, a phenyl ketone (**46**) analogue was 20-fold more active. We also examined several bioisosteres for the replacement of ethyl carboxylate. The introduction of an oxazoline (**47**), isoxazole (**54**) oxadiazole (**55**), tetrazole (**57**), phenyloxazole (**58**) all resulted in compounds with less potency than **1**. However, the methyloxazoles (**56** and **59**) resulted in compounds with improved potency. Several groups at the 4-position (**62**–**71**) were also explored while keeping the *N*-methyl citraconamide group at the 2-position. The analogues **62** through **71** had submicromolar potency with the 4-ethyl-substituted analogue **63** being the most potent compound in the series, having an IC_{50} value of 35 nM.

From this work, it is evident that a single modification at either the 2-, 4-, or 5-position of the pyrimidine can significantly improve the potency of the initial hit, **1** ($IC_{50} = 2$ μM). *N*-methylation of the 2-amino group of **1**, for example, improved potency over 6-fold (**20**, $IC_{50} = 0.3$ μM). In another example, substitution of the trifluoromethyl group at the 4-position of **1** with 2-(5-methylthienyl) improved potency 44-fold (**36**, $IC_{50} = 0.045$ μM). These independent modifications have significantly improved the potency of compound **1**, however, the potency resulting from a combination of the structural modifications is not necessarily additive. For example, *N*-methylation of the 2-amino group of compound **36** does not further improve the potency of **36**. To the contrary, the *N*-methylated analogue of **36** is less potent than **36** (**70**, $IC_{50} = 0.35$ μM).

In other analogues, the potency resulting from a combination of the structural modifications is cumulative. Substitution of the trifluoromethyl group at the 4-position of **1** with, for example, ethyl improved potency 5-fold (**28**, $IC_{50} = 0.4$ μM). In this case, methylation of the 2-amino group of compound **28** further improved its potency another 11-fold (**63**, $IC_{50} = 0.035$ μM). The cumulative versus non-cumulative SAR may be due to steric interactions between the 4-substituents and the 2-amino substituents, however, this is not yet well understood and will be further studied.

In summary, a substituted pyrimidine compound, ethyl 2-[(3-methyl-2, 5-dioxo(3-pyrrolinyl))amino]-4-(trifluoromethyl)pyrimidine-5-carboxylate (**1**), was identified from the screening of our in house diversity library. This novel compound was an inhibitor ($IC_{50} = 2$ μM) of AP-1 and NF-κB mediated transcriptional activation in Jurkat T cells and was optimized by substituting various



36 **63**

Figure 2.

groups at the 2-, 4- and 5-position of the pyrimidine ring. As a result of our SAR effort, we identified potent dual inhibitors of AP-1 and NF-κB mediated transcription with inhibitors (Fig. 2, **36**, and **63**) having an IC_{50} value of 0.045 and 0.035 μM respectively, in the Jurkat T cell assays. Evaluation of compound **63** and other compounds described herein in various in vivo studies is the next step in defining the beneficial effect of inhibiting both AP-1 and NF-κB mediated transcriptional activation.

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