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

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Abstract—In an effort to identify novel inhibitors of AP-1 and NF-κB mediated transcriptional activation, several analogues of ethyl 4-[(3-methyl-2,5-dioxo(3-pyrrolinyl))amino]-2-(trifluoromethyl)pyrimidine-5-carboxylate (**1**) were synthesized and tested in two in vitro assays. The 2-(2′-thienyl) substituted compound (**11**) was identified as the most potent in this series.

Introduction

There is now abundant evidence that T-lymphocytes orchestrate both the initiation and propagation of immune responses through the secretion of protein mediators termed cytokines.[1] These cytokines play a very important role in a number of inflammatory diseases.[2,3] In allergies and autoimmune diseases—such as asthma, psoriasis, rheumatoid arthritis, and transplant rejection—T-cell driven immune responses appear to overreact. In activated T cells, transcription factors such as the activator protein-1 (AP-1), regulate IL-2 production and production of matrix metalloproteinases, while the nuclear factor-κB (NF-κB), is essential for the transcriptional regulation of the proinflammatory cytokines IL-1, IL-6, IL-8, and TNFα.[4] Based on these observations, 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 (Fig. 1).[5]

Using automated high-throughput assays with stably transfected human jurkat T-cells, we identified a compound (**1**) from a diversity library that inhibited both AP-1 and NF-κB mediated transcriptional activation ($IC_{50} = 1$ μM) without blocking basal transcription driven by the β-actin promoter. In addition, **1** had a similar inhibitory effect on the production of IL-2 and IL-8 levels in stimulated cells. Our goal was to improve potency by exploring different substituents around the pyrimidine ring. We introduced various groups on the pyrimidine ring at 2, 4 and 6-positions of **1**. This paper describes the synthesis and the structure–activity relationship of this series of compounds.

Chemistry

The synthesis of 2- and 4-substituted analogues is shown in Scheme 1. An appropriately substituted amidine was cyclized with diethyl 2-(ethoxymethylene)propane-1,3-dioate in ethanol and sodium ethoxide to give **4**. The amidines that are not available commercially were prepared either from the corresponding nitrile or acid as shown.[6] The hydroxy group in **4** was converted to a chloro group[7] and then treated with hydrazine or methylhydrazine to give the appropriate intermediate, **5**. Treatment of the hydrazine intermediates (**5**) with appropriately substituted maleic anhydride resulted in the final products (**6**–**44**). The treatment of **10** with acetic anhydride resulted in **38**. Table 1 shows the list of compounds that were prepared with modifications at R_1, R_2, R_3, and R_4.

We also examined the importance of the carboxylate group at the 5-position. Several ester bioisosteres were introduced in place of the ethyl ester of **11**, which were

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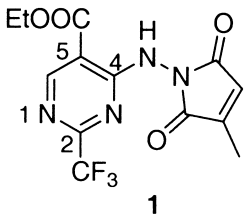

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Figure 1.

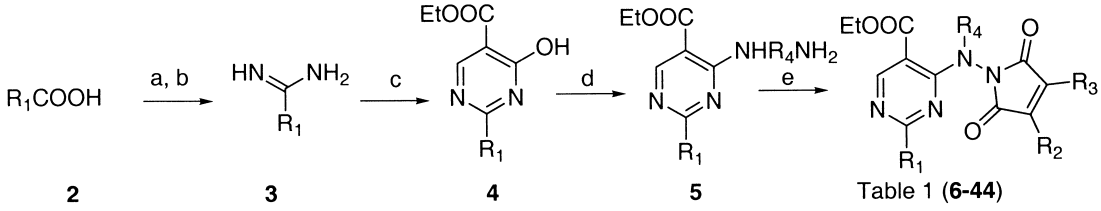
synthesized as shown in Schemes 2 and 3. The synthesis of **46** and **49** was initiated from the chloro derivative **44** (Scheme 2). The chlorine at the 4-position was replaced with a benzyl thiol followed by conversion of the ester to the isoxazole, **45**.[8]

The *S*-benzyl group in **45** was oxidized with *m*-CPBA to give a sulfone. The sulfone was treated with hydrazine followed by citraconic anhydride to give **46**. The ester group in **44** was hydrolyzed and converted to cyano analogue, **48**. The treatment of **48** with sodium azide and ammonium chloride in DMF resulted in a tetrazole ring. The tetrazole ring was methylated and *S*-benzyl group was converted to *N*-aminocitraconamide as discussed earlier to give **49**.

The synthesis of oxazole analogue **51** started from the free acid **47** (Scheme 3). The acid group was converted to an ester using a chloroacetone and to methyl oxazole **50** using acetamide and BF_3–Et_2O.[8] Compound **50** was converted to **51** using a reaction sequence for the conversion of **45** to **46**. The carboxylic acid group in **47** was converted to 2-methyl-1,3,4-oxadiazole.[9] The oxadiazole intermediate was converted to **52** using the reaction sequence for the conversion of **45** to **46**. The treatment of acid **47** with oxalyl chloride, followed by 2-aminoethanol, and then thionyl chloride resulted in dihydro oxazole **53**. The citraconamido group was introduced on to **54** as described earlier.

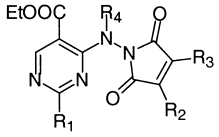
Results and Discussion

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

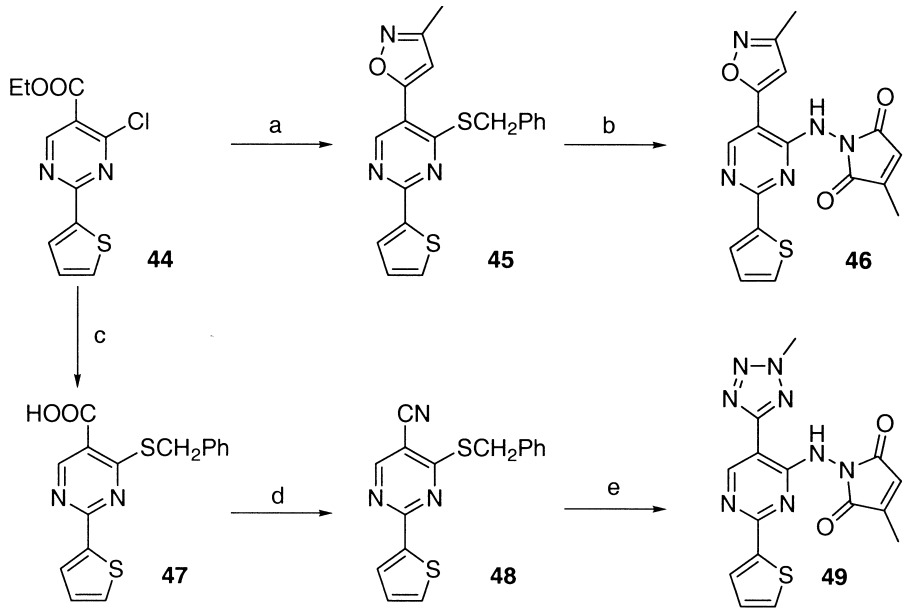


Scheme 1. (a) (i) $(COCl)_2$, DMF; (ii) NH_3, 3. $SOCl_2$; (b) (i) HCl (gas), EtOH; (ii) NH_3 (gas), EtOH; (c) Diethylethoxymethylene malonate, EtONa, EtOH; (d) (i) $POCl_3$; (ii) R_4NHNH_2; (e) Citraconic anhydride, $CHCl_3$, Δ.

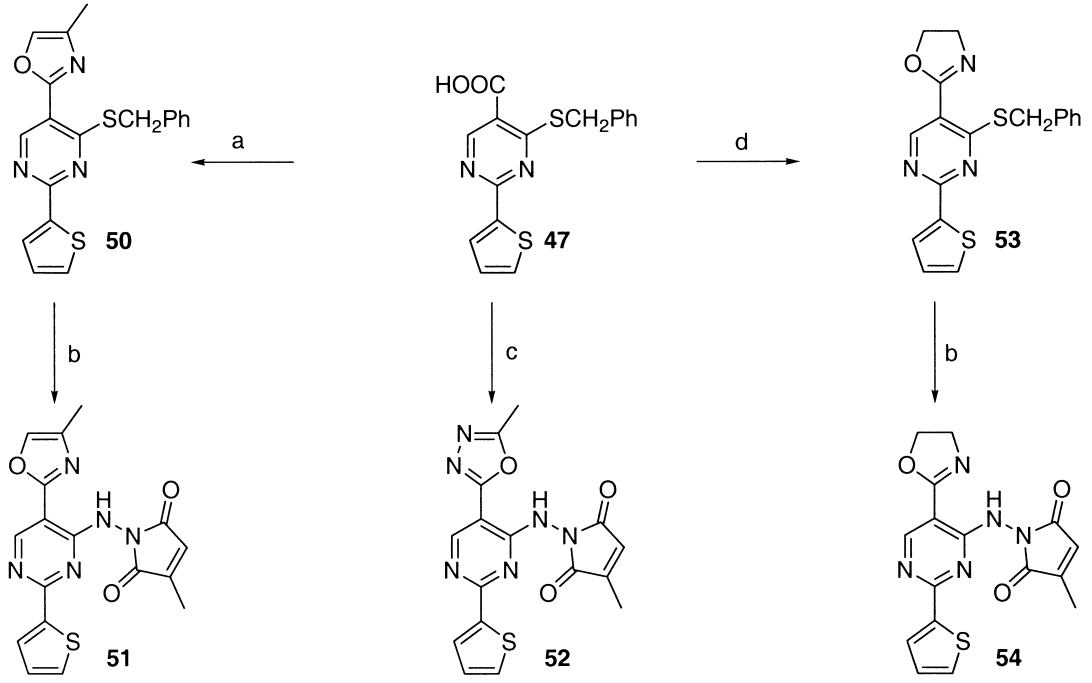
Table 1. In vitro evaluation in Jurkat T-cells of compounds with R_1 through R_4 modifications on the pyrimidine ring. IC_{50} values for the both AP-1 and NF-kB mediated transcriptional activation were the same.



No.	R_1	R_2	R_3	R_4	IC_{50}, μM	No.	R_1	R_2	R_3	R_4	IC_{50}, μM
1	CF_3	CH_3	H	H	1	**26**	5-Methyl-2-thienyl	CH_3	H	H	0.2
6	CH_3	CH_3	H	H	16	**27**	5-Chloro-2-thienyl	CH_3	H	H	0.3
7	CH_3CH_2	CH_3	H	H	10	**28**	2-Benzo-thienyl	CH_3	H	H	4
8	*t*-Bu	CH_3	H	H	1	**29**	2-Furanoyl	CH_3	H	H	0.2
9	SCH_3	CH_3	H	H	0.2	**30**	Cyclopropyl	CH_3	H	H	1
10	Ph	CH_3	H	H	0.1	**31**	2-(Cyclo-hex-2-enyl-methyl	CH_3	H	H	2
11	2-Thienyl	CH_3	H	H	0.02	**32**	3,5-Dichloro-phenyl	CH_3	H	H	0.3
12	4-Pyridyl	CH_3	H	H	6	**33**	Benzyl	CH_3	H	H	7
13	2,6-Dichloro-4-pyridyl	CH_3	H	H	2	**34**	Phenoxy	CH_3	H	H	15
14	3-Quinolinyl	CH_3	H	H	6	**35**	2-Phenyl-thio	CH_3	H	H	1
15	4-(2-Methyl-thiazolyl	CH_3	H	H	8	**36**	2-Phenyl-sulfonyl	CH_3	H	H	10
16	1-(3, 5-Dimethyl-pyrazolyl	CH_3	H	H	10	**37**	Phenyl	CH_3	H	CH_3	2
17	4-Methoxy-phenyl	CH_3	H	H	0.9	**38**	Phenyl	CH_3	H	Ac	4
18	3-Methoxy-phenyl	CH_3	H	H	5	**39**	2-Thienyl	CH_3	H	CH_3	1
19	4-Fluoro-phenyl	CH_3	H	H	0.7	**40**	4-Fluoro-phenyl	CH_3	H	Me	3
20	4-Chloro-phenyl	CH_3	H	H	0.3	**41**	CH_3CH_2	CH_3	H	Me	10
21	4-Trifluoro-methyl-phenyl	CH_3	H	H	0.4	**42**	CF_3	CH_3	CH_3	H	10
23	3-Bromo-phenyl	CH_3	H	H	0.7	**43**	CF_3	Ph	H	H	20
24	3-Nitro-phenyl	CH_3	H	H	7	**44**	CF_3	H	H	H	10
25	3-Thienyl	CH_3	H	H	0.1						



Scheme 2. (a) (i) $PhCH_2SH$, THF; (ii) Acetone oxime, *n*-BuLi, THF; (iii) H_2SO_4, THF; (b) (i) *m*-CPBA; (ii) NH_2NH_2; (iii) Citraconic anhydride, $CHCl_3$, reflux; (c) (i) $PhCH_2SH$, THF; (ii) NaOH; (d) (i) $(COCl)_2$; (ii) NH_3 (gas); (iii) $SOCl_2$, DMF; (e) (i) NaN_3, NH_4Cl, DMF; (ii) MeI, K_2CO_3; (iii) *m*-CPBA; (iv) NH_2NH_2; (v) Citraconic anhydride, $CHCl_3$, δ.

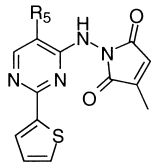


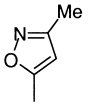
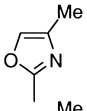
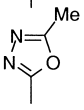
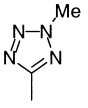
Scheme 3. (a) (i) $ClCH_2COCH_3$; (ii) Acetamide, BF_3-Et_2O, xylenes; (b) (i) *m*-CPBA; (ii) NH_2NH_2; (iii) citraconic anhydride; (c) (i) $CH_3CONHNH_2$, $POCl_3$, δ; (ii) *m*-CPBA; (iii) NH_2NH_2; (iv) Citraconic anhydride, $CHCl_3$, δ; (d) (i) $(COCl)_2$; (ii) $NH_2CH_2CH_2OH$; (iii) $SOCl_2$, EtOAc-$CHCl_3$.

binding site or a NF-κB binding site.[10] All the compounds were tested in both assays. The IC_{50} values for these compounds are shown below. Since all the compounds had similar IC_{50} values in the both AP-1 and NF-κB assays, average values are shown (Table 1).

Compound **1** had IC_{50} activity in both the cell based assays at 1 μM. The substitution of a methyl (**6**) and ethyl (**7**) in the place of trifluoromethyl group resulted in the loss of activity. However, the introduction of a *t*-butyl (**8**) group resulted in a compound with comparable activity. The *S*-methyl (**9**) and phenyl (**10**) substituents improved activity. The 2-thienyl (**11**) substituent resulted in a 50-fold improved activity. The substituted and unsubstituted heterocyclic rings (**12**–**16**) at 2-position resulted in loss of activity. Any substitution on the phenyl ring of **10** (compounds **17**–**24**) resulted in decreased activity. Similarly, introduction of a group on the thienyl ring of **11** (compounds **26**–**28**) also resulted in decreased activity. Introduction of a methyl group at R_3 (**42**), a

Table 2. In vitro evaluation in Jurkat T-cells of compounds with modifications of R_5 of the pyrimidine ring. IC_{50} values for the both AP-1 and NF-kB mediated transcriptional activation were the same.



No	R_5	IC_{50}, μM	No	R_5	IC_{50}, μM
46		0.3	**51**		0.2
48	CN	5	**52**		0.3
49		0.2	**53**		0.2

phenyl group at R_2 (**43**) or a hydrogen at R_2 (**44**) resulted in the decreased activity. Similarly, substitution of a methyl group at R_4 (**37**, **39**, **40** and **41**) resulted in the decreased activity. The substitution of an acetyl group at R_4 (**38**) also resulted in decreased activity (Table 2).

Five ester bioisoster modifications were introduced in place of the ester of **11**. A cyano analogue (**48**) was also tested. The bioisosteres **49**, **51** and **53** were 10-fold less active in the cell based assays. Analogues **46** and **52** were 15-fold less active and cyano analogue **48** was 250-fold less active. Based on the above structural activity relationship studies, a thienyl group at 2-position ring, an *N*-aminocitraconamido group at 4-position, and an ethyl ester at 5-position of the pyrimidine ring **1** are optimum substituents for the biological activity. To date we have identified a potent and novel inhibitor (**11**) of AP-1 and NF-κB mediated transcriptional activation.

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