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The Design and Synthesis of Novel Orally Active Inhibitors of AP-1 and NF-κB Mediated Transcriptional Activation. SAR of In Vitro and In Vivo Studies

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Abstract—We have developed novel orally active quinazoline analogues as inhibitors of AP-1 and NF-κB mediated transcriptional activation. Among the derivatives prepared, 1-[2-(2-thienyl)quinazolin-4-ylamino]-3-methyl-3-pyrroline-2,5-dione (**10**) showed significant activity in an adjuvant-induced arthritis rat model by reducing the swelling by 65% in the non-injected foot. The synthesis, structure–activity relationship, and in vivo activity are described.

There is abundant evidence that T-lymphocytes (T-cells) orchestrate both the initiation and propagation of immune responses through the secretion of protein mediators termed cytokines and chemokines.[1] These cytokines and chemokines play very important roles in a number of inflammatory diseases.[2] Transcription factors are regulators of inducible gene expression.[3] In activated T cells, transcription factors such as the activator protein-1 (AP-1) regulate interleukin-2 (IL-2), IL-3, and granulocyte-macrophage colony stimulating factor (GM-CSF), while the nuclear factor-κB (NF-κB), is essential for the transcriptional regulation of the proinflammatory cytokines IL-1, IL-6, IL-8 and tumor necrosis factor-α (TNFα).[4,5] Based on these observations, it appears that inhibition of AP-1 and/or NF-κB transcriptional activation in T cells may represent an attractive target in the development of novel antiinflammatory drugs. Our goal was to develop inhibitors of both AP-1 and NF-κB mediated transcriptional activation as novel therapeutic agents for treating inflammatory mediated conditions.

Earlier we reported the identification of a novel compound, **1**, that inhibited both AP-1 and NF-κB mediated transcriptional activation.[6,7] However, compound **1** had no oral activity in rat PK studies and poor Caco-2 permeability. We reasoned that the carboxylate group in **1** was responsible for the lack of oral activity. Several analogues with carboxylate bioisosteres on the 5-position of pyrimidine ring of **1** resulted in the loss of activity.[6] In order to develop potent inhibitors without a carboxylate ester function, a new class of inhibitors, **2**, was designed by introducing a fused phenyl ring on the pyrimidine ring and placing a methoxy group at the 5-position (Fig. 1) in **2**. The synthesis, structure activity relationship, and in vivo activity in an animal model are discussed below.

The synthesis of the quinazoline compounds (Scheme 1) started from the appropriately substituted 2-aminophenyl carboxylic acids (**3**).[8] The treatment of the above acids with an acid chloride followed by acetic anhydride resulted in **4**.[9] The lactone **4** was converted to a quinazoline **5** by heating with ammonium acetate at 220 °C in a steel bomb.[10] The hydroxy group at the 4-position in **5** was converted to a chloro group by treating with phosphorus oxychloride at 110 °C.[7] The chlorine at the 4-position was replaced with hydrazine.[7] The hydrazine analogue was heated at reflux with citraconic anhydride to give **6** in good yield.[7] All these compounds were evaluated in Jurkat T-cells stably transfected with either AP-1 binding site or a NF-kB binding site promoter–reporter sequence.[11]

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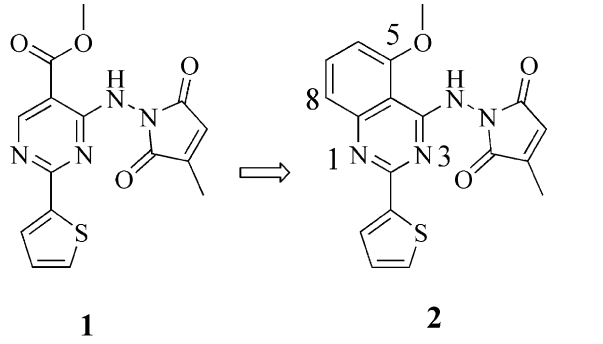
We have reported pyrimidine based analogues as inhibitors of AP-1 and NF-κB mediated transcriptional activation.[6,7] Based on our earlier studies, we selected three substituents to study at the 2-position of the quinazoline ring. We examined the effect of a 2-(2′-thienyl) (**7**), a phenyl (**8**) and a trifluoromethyl (**9**) substituion at the 2-position of the quinazoline ring (Table 1). Among the three groups examined, the compound with the 2-(2′-thienyl) group (**7**) was the most potent. We selected the 2-(2′-thienyl) and 2-trifluoromethyl substituted analogues for further lead optimization.

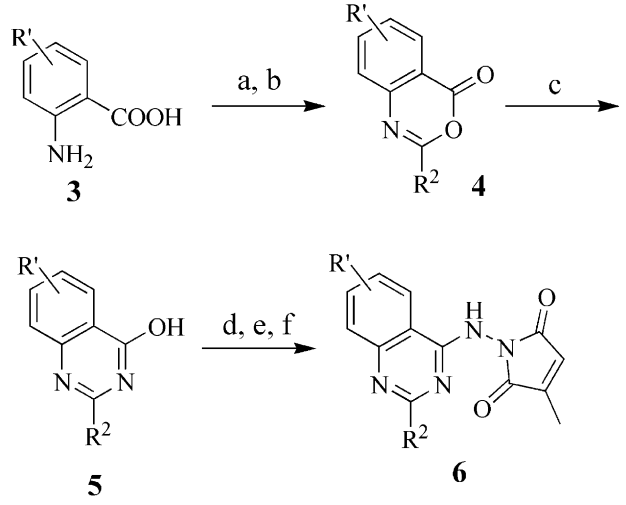
A series of 2-thienyl compounds was synthesized as shown in Scheme 1. The initial focus was on the introduction of a methoxy group on the phenyl position of the quinazoline molecule (Table 2). Compounds with a methoxy group at the 5-position (**10**) and a methoxy at the 6-position (**11**) had comparable potency. However, a methoxy group at the 7-position (**12**) or the 8-position (**13**) resulted in the loss of 2- to 4-fold in potency. The introduction of two (**14**) and three methoxy (**15**) groups resulted in compounds that were 10-fold less potent than **10**. The introduction of electron withdrawing atoms such as fluorine at the 5-position (**16**) or chlorine at the 6-position (**17**) resulted in a 4- to 5-fold loss in potency, compared to **10**. The introduction of an *N*-morpholine group (**19**) at the 7-position resulted in a 10-fold drop in potency compared to the 7-methoxy analogue (**12**). However, a compound with an *N*,*N*-dimethylamino group at the 7-position (**20**) had comparable potency to the 7-methoxy analogue (**12**) and was 5-fold less potent than **10**. The hydrochloride salts of both the morpholino compound (**19**) and the dimethylamino analogue (**20**) had better water solubility than **10**.

A similar trend was observed with the trifluoromethyl series (Table 3). A 5-methoxy analogue (**21**) and a 6-methoxy analogue (**22**) were 10-fold more potent than the unsubstituted analogue (**9**). However both **21** and **22** were approximately 10-fold less active than **10** and **11**, respectively. The substitution of an alkyl group at the 5-position resulted in a 40-fold drop in potency. However, the substitution of SMe at the 6-position resulted only in a 3-fold drop in potency. The introduction of methylsulfoxide (**25**) and methylsulfone (**26**) at the 6-position resulted in a 30-fold and a 300-fold drop in potency, respectively. An hydroxy group at the 6-position (**27**) in the place of OMe (**22**) resulted in very small



1 **2**

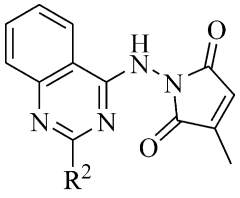
Figure 1.



3 **4** **5** **6**

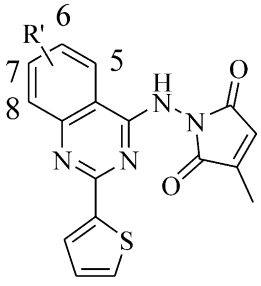
Scheme 1. (a) R^2COCl, THF, rt, 2 h; (b) $(CH_3CO)_2O$, reflux, 1 h, 82–99% from **3**; (c) CH_3COONH_4, 220 °C, steel bomb, 7 h, 81–97%; (d) $POCl_3$ (10 equiv), reflux, 3 h, 84–94%; (e) hydrazine (2.2 equiv), THF, rt, 1 h; (f) citraconic anhydride (1.1 equiv), chloroform, Dean-Stark receiver, reflux, 12 h, 82–98% combined yield for steps e and f.

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



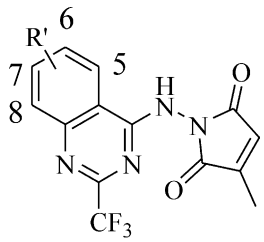
No	R^2	IC_{50}, μM
7	(2′Thienyl)	0.06
8	Phenyl	0.1
9	CF_3	0.1

Table 2. Inhibition of AP-1 and NF-κB mediated transcriptional activation in Jurkat T-cells



No.	R′	IC_{50}, μM
10	5-OMe	0.008
11	6-OMe	0.003
12	7-OMe	0.02
13	8-OMe	0.05
14	6,7-di-OMe	0.05
15	6,7,8-tri-OMe	0.04
16	5-F	0.05
17	6-Cl	0.02
18	5-Me	0.02
19	7-(*N*-morphinoyl)	0.2
20	7-(NMe_2)	0.04

Table 3. Inhibition of AP-1 and NF-κB mediated transcriptional activation in Jurkat T-cells



No.	R′	IC_{50}, μM
21	5-OMe	0.01
22	6-OMe	0.03
23	5-Me	0.4
24	6-SMe	0.08
25	6-SOMe	1
26	6-SO_2Me	9
27	6-OH	0.1
28	7-(1-Piperidyl)	0.2
29	7-(NMe_2)	0.02

Table 4. Caco-2 permeability coefficient values

No.	Pc, cm/s
Caffeine	$4.4\pm0.1\times10^{-5}$
10	$1.41\pm0.4\times10^{-5}$
11	$1.62\pm0.2\times10^{-6}$
20	$1.41\pm0.6\times10^{-6}$
21	$1.49\pm0.3\times10^{-6}$
29	$1.68\pm0.3\times10^{-6}$

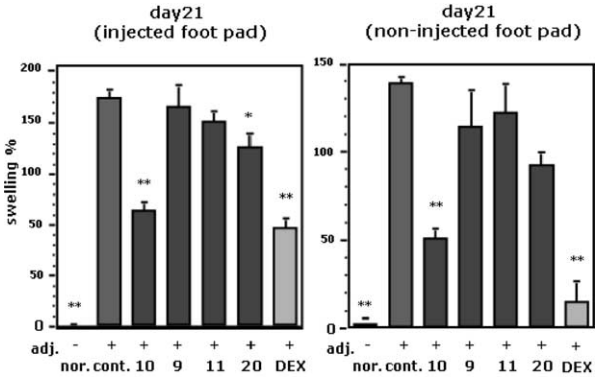


Figure 2. Effects of compounds on footpad swelling in adjuvant arthritic rats. Compounds were administered 30 mg/kg, po. Each point represents the mean ± S.E. of 5 animals per group. *, **: Significantly different from the control, $p<0.01$, respectively.

drop in potency. Two analogues with a basic nitrogen at the 7-position were prepared. The 7-(1-piperidyl) analogue (**28**) was 20-fold less active than **21** but the 7-(*N*,*N*-dimethyl) analogue (**29**) was comparable in potency to **21**.

We selected a set of 5 compounds to study their Caco-2 permeability.[12] As shown in Table 4, compound **10** showed acceptable permeability. However, the rest of the analogues were less permeable than **10**. We further examined these compounds in the adjuvant induced arthritis rat-model.[13] On day 21, the swelling on both injected foot and non-injected foot pad went down significantly in the animals treated with compound **10** (Fig. 2). However, the rest of the compounds (**9**, **11**, **20**) caused modest reduction in swelling. In conclusion, we have developed an inhibitor of AP-1 and NF-κB mediated transcriptional activation that showed efficacy in an animal disease model. This compound (**10**) showed significant activity in the adjuvant-induced arthritis rat model by reducing the swelling by 65% in the non-injected foot. Further evaluation of **10**[14] is in progress.

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11. **NF-κB Assay:** Human Jurkat T-cells stably transfected with an NF-kB binding site (from the MHC promoter) fused to a minimal SV-40 promoter driving luciferase were used in these experiments.[1] Cells were counted, resuspended in fresh medium containing 10% serum-plus at a density of 1×10^6 cells/mL, and plated in 96-well round-bottom plates (200 μL per well) 18 h prior to running the assays. Compounds dissolved in 0.2% DMSO/H_2O at the appropriate concentrations were then added to the microtiter plates containing the cells, and the plates were incubated at 37 °C for 0.5 h. To induce transcriptional activation, 50 μg/mL of phorbol 12-myristate-13-acetate (PMA) and 1 ug/mL of phytohemagglutin (PHA) were added to each well, and the cells were incubated for an additional 5 h at 37 °C. The plates were centrifuged at 2200 rpm for 1 min at room temperature followed by the removal of the media; 60 μL of cell lysis buffer was added to each well, and cells were lysed 0.25 h; 40 μL of each cell lysate was transferred to a black 96-well plate, and 50 μL of luciferase substrate buffer was added. Luminescence was immediately measured using a Packard TopCount. The results are expressed as IC_{50} values where the IC_{50} value is defined as the concentration of compound required to reduce luciferase activity to 50% of control values.
AP-1 Assay: The AP-1 assay was run as described above for NF-kB except that the Jurkat T-cells were stably transfected with a plasmid that contained an AP-1 binding site from the collagenase promoter driving luciferase expression. In addition, the concentration of PMA and PHA were 5 μg/mL and 1 μg/mL, respectively.
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13. Female Lewis rats, 8 weeks of age, were purchased from Charles River, Japan. After acclimatization, the rats were randomly assigned into 12 groups ($n=5$ per group) according

to treatment received. Rats in 11 groups were induced with arthritis by injecting 100 μg of heat-inactivated *Mycobacterium butyricum* (Difco, USA) in 100 μL of liquid paraffin in the right hind paw (Day 0), and the rest group was used as a healthy control group. Compound **9**, **10**, **11** and **20** were suspended in 0.5% carboxymethylcellulose solution containing 0.1% Tween80 and saline for 30 mg/kg, po administration. Dexamethsone 21-phosphate (DEX, a antiinflammatory steroid from Sigma), a positive reference compound, was suspended in 0.5% carboxymethylcellulose solution and administered to rats at a dose level of 0.1 mg/kg, po All compounds and vehicles were administered to arthritic rats once daily for 21 days. The degree of arthritis was determined by measuring hind-paw volume using a plethysmometer (TK-lO5, Unicom, Tokyo) on the Day 0 (immediately before adjuvant injection), 3, 7, 10, 14, 17, and 21.

Statistical Analysis: All values are means + SEM unless otherwise stated. Statistically significance was determined by the Tukey-Kamer procedure using SuperANOVA software (Abacus Concepts, USA). The level of significance was $p < 0.05$.

14. 1-[2-(2-thienyl)quinazolin-4-ylamino]-3-methyl-3-pyrroline-2,5-dione (**10**). Mp 199--200 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.40 (s, 1H), 7.82 (dd, $J = 1.5$ and 3.9 Hz, 1H), 7.56 (t, $J = 8.1$ Hz, 1H), 7.42 (m, 2H), 7.07 (dd, $J = 3.6$ and 1.2 Hz, 1H), 6.62 (m, 2H), 3.83 (m, 3H), 2.26 (d, $J = 1.8$ Hz, 3H); IR (KBr, cm^{-1}) 3099, 2942, 1734, 1576, 1500, 1315, 1259, 1086, 731; EIMS m/z 366 (M^+);