

Small Molecule Inhibitors Targeting Activator Protein 1 (AP-1)

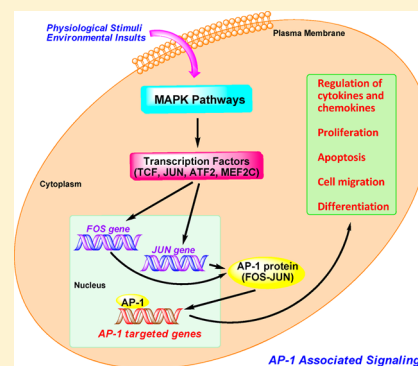
Miniperspective

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ABSTRACT: Activator protein 1 (AP-1) is a pivotal transcription factor that regulates a wide range of cellular processes including proliferation, apoptosis, differentiation, survival, cell migration, and transformation. Accumulating evidence supports that AP-1 plays an important role in several severe disorders including cancer, fibrosis, and organ injury, as well as inflammatory disorders such as asthma, psoriasis, and rheumatoid arthritis. AP-1 has emerged as an actively pursued drug discovery target over the past decade. Excitingly, a selective AP-1 inhibitor T-5224 (**51**) has been investigated in phase II human clinical trials. Nevertheless, no effective AP-1 inhibitors have yet been approved for clinical use. Despite significant advances achieved in understanding AP-1 biology and function, as well as the identification of small molecules modulating AP-1 associated signaling pathways, medicinal chemistry efforts remain an urgent need to yield selective and efficacious AP-1 inhibitors as a viable therapeutic strategy for human diseases.



1. INTRODUCTION

Activator protein 1 (aka activating protein 1, AP-1) is a critical transcription factor that participates in a wide range of cellular processes including proliferation, apoptosis, differentiation, survival, cell migration, and transformation. AP-1 has emerged as an actively pursued drug discovery target and has received particular attention over the past 2 decades with a resurgence of interest in recent years (Figure 1). Accumulating evidence suggests that AP-1 plays an important role in several severe disorders including cancer, fibrosis, and organ injury, as well as inflammatory disorders such as asthma, psoriasis, rheumatoid arthritis, and transplant rejection.^{1–4} Despite the great therapeutic potential of this target and the tremendous

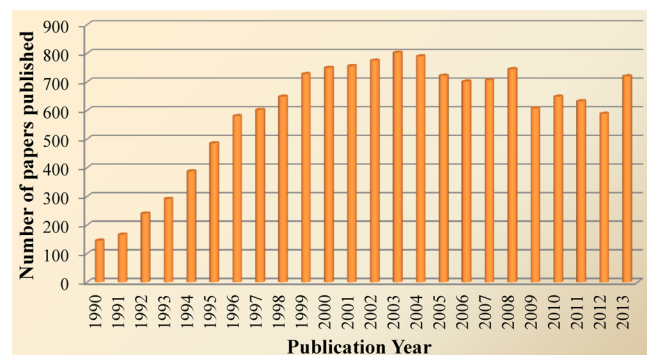


Figure 1. Number of papers published between 1990 and 2013 according to recent PubMed search using “AP-1”.

academic and industrial efforts dedicated to it, only one selective AP-1 inhibitor has been advanced into human clinical trials. 3-[5-[4-(Cyclopentyloxy)-2-hydroxybenzoyl]-2-[(3-hydroxy-1,2-benzisoxazol-6-yl)methoxy]phenyl]propionic acid (T-5224, **51**),⁵ a novel AP-1 inhibitor co-developed by Toyama Chemical and Kitasato University, has proven to prevent joint destruction, pannus formation, and osteoclastogenesis in collagen-induced arthritis (CIA) in rats. **51** shows good promise as a new drug for the treatment of arthritis and is currently in phase II human clinical trials in Japan. Additionally, **51** is under investigation for other inflammatory diseases in which AP-1 is involved. This review is structured to provide the readers with a brief summary of AP-1 family proteins, structures, functions, AP-1 associated signaling pathways, and their roles in various human diseases, as well as the development of AP-1 inhibitors and hit-to-lead optimizations over the past 2 decades from a medicinal chemistry perspective.

2. AP-1 FAMILY PROTEINS, STRUCTURES, AND FUNCTIONS

The AP-1 family of transcription factors is composed of homodimers and heterodimers of Jun (v-Jun, c-Jun, JunB, and JunD), Fos (v-Fos, c-Fos, FosB, Fra1, and Fra2), ATF (ATF2, ATF3/LRF1, B-ATF, JDP1, and JDP2), and MAF (c-Maf, MafB, MafA, MafG/F/K, and Nrl) protein families,^{6,7} which are characterized by highly conserved dimeric basic leucine zipper (bZIP) DNA-binding domains. The leucine zipper is a

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structural motif that forms an extended α -helix in which every seventh amino acid is a leucine.⁷ The carboxy-terminal regions of α -helices align to form parallel “coiled coils”, while the amino-terminal regions make base-specific contacts with DNA in the major groove (Figure 2, c-Fos/c-Jun, PDB code 1Fos).⁸

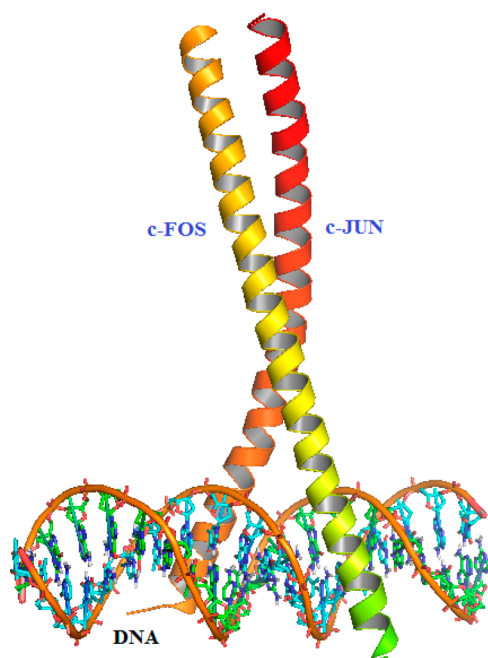


Figure 2. Structures of AP-1 (c-Fos/c-Jun, PDB code 1Fos).

There are a number of crystal structures of AP-1 protein domains and complexes available from the Protein Data Bank, which have significantly facilitated the understanding of the AP-1 family proteins and their structural diversities.

These multiple family members are expressed in a cell- and stage-dependent manner during development and mediate the transcription of specific genes at different levels.^{7,9} Among them, the Jun and Fos subfamilies are the most studied and the major AP-1 proteins. Although members of the Jun and Fos families share a high degree of structural homology, the individual AP-1 dimers exert significant differences in their DNA binding affinity and their capability of activating or suppressing gene expression, suggesting specific functions in gene regulation for individual AP-1 dimers.² The Jun proteins can both homo- and heterodimerize with members of Fos and ATF subfamilies, whereas Fos protein can only heterodimerize with Jun proteins rather than homodimerize among themselves. Jun-Jun and Jun-Fos dimers prefer to bind to a heptamer consensus sequence known as the TPA-responsive element (TRE, 5'-TGA(C/G)TCA-3'), whereas Jun-ATF dimers or ATF homodimers preferentially bind to a different consensus sequence known as the cAMP-responsive element (CRE, 5'-TGACGTCA-3').¹⁰ In addition, AP-1 proteins can also interact with non-bZIP proteins, including the p65 subunit of NF- κ B, CBP/p300, and Rb, further expanding the combinatorial diversity of AP-1 family proteins and the spectrum of regulated genes.¹¹

A variety of AP-1 associated biological functions in development and disease have been revealed from extensive

Table 1. Functions of Jun and Fos proteins

phenotype		Transgene/Promoters	affected organs/cell types
c-Fos/H2K ^b	Osteosarcoma	Bone/osteoblasts	
FosB/H2K ^b	None	Bone	
Δ FosB/TCR β	Impaired T cell differentiation	Thymus/immature thymocytes	
Δ FosB/NSE	Osteosclerosis	Bone/osteoblasts	
Fra1/H2K ^b	Osteosclerosis	Bone/osteoblasts	
Fra2/CMV	Ocular malformations	Anterior eye structure	
Fra2 ^a /H2K ^b	Increased bone mass/fibrosis	Bone/internal organs/skin	
c-Jun/H2K ^b	None	None	
JunB/ubiquitin C	Increase bone mass	Not defined	
JunB/CD4	Enhanced Th2 maturation	Thymus/CD4 thymocytes	
JunD/Ubiquitin C	Peripheral T cells and B cells reduced	Lymphocytes	
Knock-Out			
c-Fos	Osteopetrosis	Bone/osteoclasts	
FosB	Nurturing defect	Brain/hypothalamus	
Fra1	Embryonic lethality (E9.5)	Extra-embryonic tissue/yolk sac; placenta/labyrinth layer	
Fra2	Lethal at birth	Bone/osteoclasts	
c-Jun	Embryonic lethality (E12.5)	Live/hepatoblasts; heart/outflow tract	
JunB	Embryonic lethality (E8.5-10)	Extra-embryonic tissue/yolk sac, giant trophoblasts; placenta/labyrinth layer	
JunD	Male sterility	Testis/spermatogenesis	
Conditional Knock-Out			
c-Jun/Alfp-cre	Impaired liver regeneration	Liver/hapocytes	
c-Jun/Col2a1	Scoliosis	Bones/notochordal cells	
c-Jun/K5-cre	Eye open at birth reduced tumors	Keratinocytes	
c-Jun/Nestin-cre	Axonal regeneration defect	Central nervous system/motoneurons	
JunB/MORE-cre	Osteopenia	Bone/osteoclasts, osteoblasts	
c-Fos/Nestin-cre	Learning defects	Brain/hippocampal neurons	
Fra1/MORE-cre	Osteopenia	Bone/osteoblasts	
JunB + c-Jun/K5-creER ^T	Psoriasis, psoriatic arthritis	Skin, joint/keratinocytes	

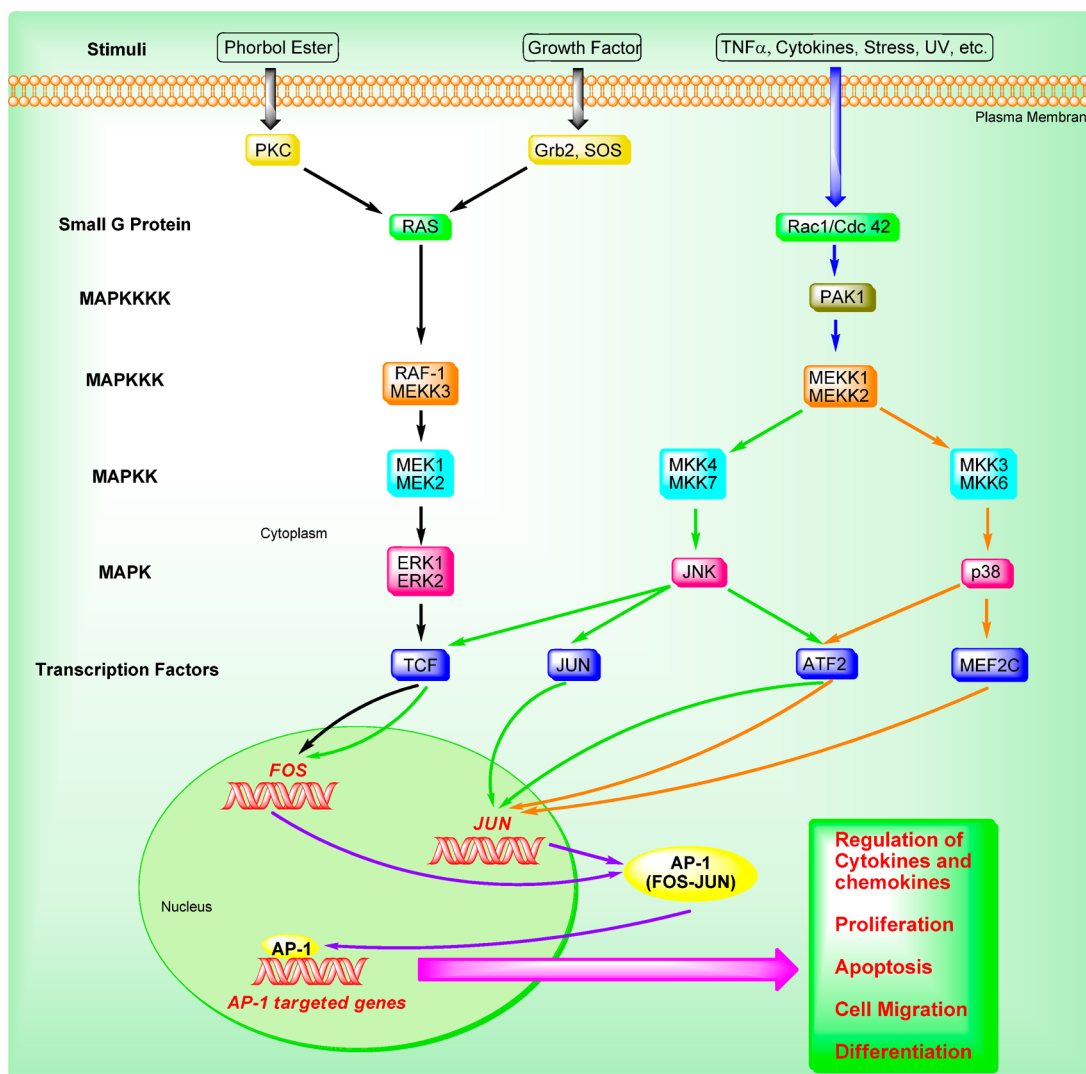


Figure 3. AP-1 associated signaling pathways.

analyses of mice and cells harboring genetic modifications of distinct *Fos* and *Jun* genes. As summarized in Table 1,^{12–14} many important insights have been provided through gain-of-function and loss-of-function experiments using transgenic and embryonic stem (ES) cell technology.

3. AP-1 SIGNALING PATHWAYS

AP-1 activity is induced by a plethora of physiological stimuli and environmental insults, such as the phorbol ester tumor promoter TPA, growth factors, neurotransmitters, polypeptide hormones, cell–matrix interactions, cytokines, UV irradiation, and bacterial and viral infections.^{2,15,16} AP-1 protein is primarily regulated at the level of both *Jun* and *Fos* gene transcription involving mitogen-activated protein kinases (MAPKs) pathways and by post-translational modification via phosphorylation and dephosphorylation.¹⁵

As depicted in Figure 3, MAPK cascades consist of three- or four-tiered signaling modules in which the MAPK is activated by a MAPK kinase (MAPKK), which in turn is activated by a MAPK kinase kinase (MAPKKK). The MAPKKK itself is activated by a small G-protein such as Ras, either directly or via another upstream kinase.¹⁷ Among these, the JNKs and ERKs, together with the later discovered p38s, constitute three

separate groups of MAPKs. The JNKs are activated by the MAPKKs (MKK4 and MKK7). The ERKs are activated by the MEK1 and MEK2, and the p38 is activated by the MKK3 and MKK6.¹⁸ After MAPKs are activated, they are able to regulate downstream transcription factors that induce the transcription of *Fos* and *Jun* genes, thereby increasing the expression of AP-1 complexes. The expression of *Fos* is induced by TCFs, which are activated through phosphorylation by the p38, JNKs, and ERKs. The expression of *Jun* is induced by MEF2C, ATF2, and JUN, which are activated through phosphorylation by the p38 and JNK.¹⁸ Once AP-1 and other associated cellular factors are activated, they can participate in regulating altered gene expression, cellular proliferation, apoptosis, differentiation, and migration in response to cytokines and growth factors, noxious stimuli, and oncogenic transformation.

In addition, various kinases via post-translational phosphorylation also regulate AP-1 activity, including its transactivating potential, DNA-binding capacity, and the stability of AP-1 components; for instance, casein kinase II (CKII), glycogen synthase kinase-3 β (GSK-3 β), and ribosomal S6 kinase 2 (RSK2). Recently, it has also been shown that the AP-1 protein can be regulated and activated by several other mechanisms, including genetic interaction with other oncoproteins or

ancillary proteins, messenger RNA turnover, and protein stability.²

4. AP-1 AS A POTENTIAL TARGET FOR HUMAN DISEASES

4.1. AP-1 and Inflammatory Diseases. Inflammation is a part of the complex physiological response of the human body to harmful stimuli, such as pathogen invasion, irritants, and tissue injury. Inflammation is widely recognized as the body's attempt at self-protection and a beginning of the healing process. In the course of inflammation, immune cells of the innate and/or adaptive immune system are activated, and a variety of different cytokines and chemokines, which are predominantly regulated by AP-1 and other transcription factors including NF- κ B, NFATs, and STATs, are recruited to the site of inflammation. Inappropriate activation of the immune system, such as in the case when inflammatory cells and proteins attack and destroy healthy tissue, can result in the overproduction of immune cells, inflammatory cytokines, and tissue-destructive enzymes, thereby giving rise to inflammatory disorders including osteoarthritis, periodontal disease, rheumatoid arthritis, multiple sclerosis, asthma, inflammatory bowel disease, and psoriasis. Interestingly, many cytokine genes are cooperatively regulated by a transcription factor complex consisting of AP-1 and NFAT. AP-1 is a proinflammatory element that is believed to directly control the expression of the cytokines such as tumor necrosis factor α (TNF- α), interleukin 1 (IL-1), interleukin 2 (IL-2), IFN γ , GM-CSF, and matrix-degrading matrix metalloproteases (MMPs) such as collagenase 1 and stromelysin at the level of mRNA synthesis by directly binding their promoter AP-1 binding motifs.^{7,19–31} Moreover, AP-1 also induces many of the genes that are dependently regulated by NFAT.³²

Increasing evidence supports that AP-1 plays a vital role in the initiative and development of inflammatory disorders. It is believed that AP-1 activation is a necessary step in the chain of events that lead to joint erosion. Systematic administration of an AP-1 decoy oligodeoxynucleotide to competitively inhibit AP-1 DNA binding was found to reduce arthritic joint destruction in mice with collagen-induced arthritis.²⁷ Moreover, increased activation and expression of AP-1 have been shown in the airway of asthmatic patients.^{33,34} The gene for Muc5B, which contains a putative AP-1 consensus site in its promoter, is responsible for the airways mucus production.³⁵ Furthermore, in psoriatic lesions, epidermal keratinocytes have decreased expression of *JunB* (19p13.2), a gene localized in the psoriasis susceptibility region PSORS6 (19p13).³⁶ Both human and inducible mouse models have demonstrated that down-regulation of *JunB* in keratinocytes is one initiating event in the etiology of psoriasis that is characterized by increased cell proliferation and deregulated cytokine expression.^{37–39}

Taken together, inhibition of AP-1 may act as a promising therapeutic strategy to provide the benefit of treating the underlying inflammatory process and reducing the production of inflammatory cytokines and chemokines, which play a key role in inflammatory disorders.

4.2. AP-1 and Cancer. In 1982, murine c-Fos proteins were first identified as the viral oncoproteins v-Fos in the Finkel-Biskis-Jenkins osteosarcoma virus and showed increased transforming activity.^{40–42} Later, mouse c-Jun protein was also first found in its viral counterparts v-Jun in the avian sarcoma virus 17.⁴² Other Jun (*JunB* and *JunD*) and Fos (*FosB*, *Fra1* and *Fra2*) proteins have also been identified as

oncoproteins, all of which are components of AP-1. Several AP-1 proteins such as c-Fos, FosB, and c-Jun have potent transactivation domains,¹² which are identified by their ability to induce target-gene transcription and efficiency in transforming cells in culture. Other AP-1 proteins have either a weak transforming activity (*Fra1* and *Fra2*^{43,44}) or no transforming activity (*JunB* and *JunD*⁴⁵) due to the lack of potent transactivation domains. Nevertheless, all of these AP-1 proteins are actively involved in tumor development and progression.

Numerous studies have shown that the AP-1 family of transcription factors play a crucial role in proliferation, apoptosis, angiogenesis, oncogene-induced transformation, and invasiveness (Table 2) and are involved in many cancers

Table 2. AP-1 Target Genes in Tumor Development and Suppression

main regulator	gene product	activity
c-Fos (up-regulates)	DNMT1	DNA methylation ⁶⁰
	FASL	Stimulates apoptosis ⁶¹
	VEGFD	Angiogenesis ⁶²
	MMP1, MMP3, CD44,	Invasiveness ^{63–65}
c-Jun (up-regulates)	EGFR, HB-EGF, GM-CSF, KGF,	Stimulates proliferation ⁶⁶
	FASL, BIM	Stimulates apoptosis ⁶⁷
	BCL 3	Inhibits apoptosis ⁶⁸
	Proliferin	Angiogenesis
	CD44	Invasiveness ⁶⁹
c-Jun (down-regulates)	WAF-1, p53, INK4A,	Inhibits proliferation ^{52,53,70,71}
	Cyclin Ds, E2F2, DP1	
	P53, FAS	Stimulates apoptosis ⁷²
JunB (up-regulates)	EGFR	Stimulates proliferation ⁷³
	INK4A	Stimulates apoptosis ⁷⁴
	Proliferin	Angiogenesis ⁷⁵
JunB (down-regulates)	GM-CSF, KGF, cyclin D1	Stimulates proliferation ^{71,76,77}
	BCL 3, BCL-XL	Inhibits apoptosis ⁵⁶
JunD (down-regulates)	ARF	Inhibits proliferation ⁷⁸
Fra1 (up-regulates)	uPA, uPAR	Angiogenesis ⁷⁹
	MMP3, MMP1	Invasiveness ⁸⁰

including breast, ovarian, liver, skin, bone, lung, endometrial, and colorectal tumors.¹⁸ When widely overexpressed in mice, c-Fos causes osteosarcoma formation by the transformation of chondroblasts and osteoblasts, which identifies these two cell types as cellular targets of c-Fos-induced tumorigenesis.^{46,47} It has also been revealed that c-Fos is required for breast cancer cell growth.⁴⁸ Although c-Jun is variably expressed in human breast tumors with only approximately 20–40%, c-Jun overexpression in MCF-7 breast cancer cells can produce highly invasive and hormone-resistant tumors.^{49,50} It is found that blockade of c-Jun with the expression of a dominant-negative c-Jun (TAM67) reduces the expression of cyclin Ds, E2Fs, and DP1, resulting in suppressed cell proliferation in vitro and in vivo.^{51,52} Conditional inactivation of c-Jun by overexpressing TAM67 in mammary epithelial cells, basal keratinocytes, or the liver reduces c-Jun activity and interferes with the development of transgene- or chemical-induced ER-negative mammary tumor, papillomas, and liver tumors, respectively,^{51,53,54} suggesting that c-Jun is an important target in the development of ER-negative breast cancer, skin and liver tumors. In addition, overexpression of *Fra1* and *Fra2* in

transgenic mice can lead to the development of lung tumors and epithelial tumors, respectively.⁵⁵ Furthermore, loss of the JunB transcription factor induces a myeloproliferative disease (MPD) arising from the hematopoietic stem cell (HSC) compartment.⁵⁶ JunB inactivation has been observed in a spectrum of human myeloid malignancies including CML,⁵⁷ and down-regulation of JunB expression has also been found in the HSC compartment of patients with acute myeloid leukemia.⁵⁸ Therefore, JunB protects against myeloid malignancies by limiting hematopoietic stem cell proliferation and differentiation without affecting self-renewal.⁵⁹

In summary, accumulating studies support that increased expression of AP-1 is associated with a variety of human cancers, and AP-1 is actively implicated in tumor progression and development. The available findings suggest that AP-1 may act as a promising target for cancer prevention and therapy and that AP-1 inhibitors may have great potential to be developed as effective drugs for oncogene-induced transformation, premalignant lesions, and tumor cell growth.

4.3. AP-1 and Other Human Diseases. Of the several potential therapeutic indications by modulating AP-1 activity, inflammation and cancer represent the two most promising major areas. Moreover, compelling evidence using genetically modified mice has provided novel insights into the fundamental functions of AP-1 and shown that up-regulation or down-regulation of AP-1 expression also has a significant contribution in the progression or initiation of various other human diseases, including hepatitis, pulmonary fibrosis, atherosclerosis, cardiovascular diseases, and Parkinson's disease (PD).

4.3.1. Hepatitis and Liver Injury. c-Jun and JunB are strongly expressed in the liver of human and murine with hepatitis. In hepatocytes, the dimeric transcription factor c-Jun is a major mediator of cell survival during hepatitis (inflammation of the liver). Mice lacking c-Jun in hepatocytes display increased liver cell death and mortality upon administration of Con A, which induces liver inflammation and injury. Wagner and colleagues⁸¹ found that this phenotype was caused by impaired expression of inducible nitric oxide synthase (NOS2), a direct transcriptional target of c-Jun, and reduced production of hepatoprotective nitric oxide (NO). Moreover, increased hepatotoxicity in mutant mice is likely caused by hypoxia and oxidative stress and can be pharmacologically rescued by liver-specific NO delivery. Thus, c-Jun is hepatoprotective during acute hepatitis by regulating NOS2/NO expression and thus functionally antagonizes the cytokine-induced cell death-promoting functions of JNK during hepatitis.⁸¹ Surprisingly, c-Jun does not seem to be critical for immune response in hepatitis. Mice specifically lacking JunB in hepatocytes are also found to display a mild increase in Con A-induced liver damage. Using loss-of-function mouse models for JunB, Wagner and co-workers⁸² have subsequently demonstrated that JunB promotes cell death during acute hepatitis by regulating IFN- γ production in NK and NKT cells and thus functionally antagonizes the hepatoprotective function of c-Jun in hepatocytes.

4.3.2. Pulmonary Fibrosis. In transgenic mice, ectopic expression of Fra 2 in various organs results in generalized fibrosis with predominant manifestation in the lung, possibly by linking vascular remodeling and fibrogenesis.⁸³ Strong expression of Fra 2 was also observed in human samples of idiopathic and autoimmune-mediated pulmonary fibrosis.⁸³ Fra2 has been considered as a contributing pathogenic factor of pulmonary fibrosis in humans. However, different from Fra2,

Fra1 plays a protective role in lung fibrosis⁸⁴ and modulates early profibrotic cellular responses.⁸⁵ Fra1 mediates antifibrotic effects through the modulation of expression of proinflammatory, profibrotic, and fibrotic gene both in vitro and in vivo.⁸⁴ Thus, either Fra1 or Fra2 transcription factors may act as potential targets for pulmonary fibrosis, a progressive disorder with poor prognosis and treatment.

4.3.3. Atherosclerotic Disease. In vitro and in vivo animal studies implicate AP-1 as a critical common inflammatory transcription factor correlated in the initiation and progression of vascular dysfunction and atherogenesis.^{86–90} Lindeman and colleagues⁹¹ have demonstrated that abundant AP-1 activation is associated with all stages of atherosclerosis by performing systematic histological evaluation. Lai and colleagues⁹² have revealed that irbesartan, an angiotensin II receptor antagonist, may modulate inflammation-based atherosclerotic diseases through a cell-mediated mechanism involving suppression of human T-lymphocytes activation via down-regulation of AP-1 activity. Hence, AP-1 inhibition may represent a promising strategy to prevent progression of atherosclerotic disease.

4.3.4. Other Cardiovascular Diseases. Early immediate up-regulation of AP-1 in response to cardiac hypertrophic stimuli was reported in the 1990s.^{93–96} Two members of the AP-1 family of transcription factors, JunD and Fra1, have been found to play an important role in regulation of heart growth during hypertrophic response.^{97,98} JunD, the only Jun protein constitutively and highly expressed in mammalian heart, can attenuate phenylephrine-mediated cardiomyocyte hypertrophy by inhibiting AP-1 transcriptional activity and may effectively function as an endogenous dominant negative regulator, buffering against extensive hypertrophic growth of cardiomyocytes in response to pathophysiological stress.⁹⁹ In several in vitro studies, c-Jun and c-Fos have been suggested to be required for induction of fetal gene expression and cardiomyocyte hypertrophy in response to different stimuli.^{100–105} In fact, both Jun and Fos are not essential for postnatal cardiac hypertrophy as well as heart growth in response to mechanical pressure overload from a genetic evidence in vivo.¹⁰⁶ Remarkably, however, deletion of Jun but not Fos can result in progressive myocardial fibrosis, cardiomyocyte apoptosis, and changes in sarcomeric organization.¹⁰⁶ Additionally, the AP-1 components FosB and JunB regulate the intrinsic matrix metalloproteinase 2 (MMP-2) promoter in vivo following ischemia–reperfusion injury.¹⁰⁷ MMP-2, which influences ventricular performance, is a central component of the response to injury in the heart.

4.3.5. Parkinson's Disease (PD). AP-1 is involved in the neuropathology of PD patients, whose phenotype can be closely mimicked by MPP⁺-induced neurotoxicity in vitro. Using an S-type human neuroblastoma cell line (SH-EP1) as a model, Feng and colleagues¹⁰⁸ investigated the involvement of NF- κ B and AP-1 pathways in MPP⁺-induced neurotoxicity. Besides NF- κ B, JNK and c-Jun are also activated upon MPP⁺ stimulation. Inhibition of c-Jun activation, by a dominant negative c-Jun or c-Jun inhibitor such as curcumin, can significantly attenuate MPP⁺-mediated cell death, suggesting that c-Jun activation is proapoptotic.¹⁰⁸ Thus, down-regulating the activation of NF- κ B, JNK, and c-Jun may represent a new strategy for the treatment of PD.

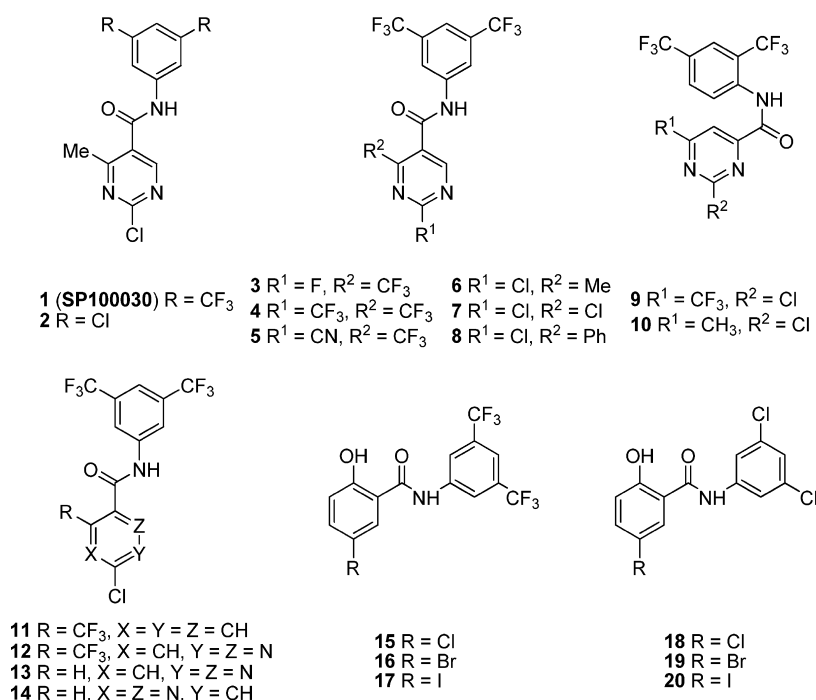


Figure 4. Chemical structures of **1** and its representative analogues **2**–**20**.

5. DEVELOPMENT OF SMALL MOLECULES TARGETING AP-1

As AP-1 plays important pathophysiological roles in various human conditions, AP-1 inhibitors are currently under intense development preclinically and clinically in many therapeutic areas, especially for inflammatory disease and cancers. Several core molecular scaffolds have been identified to be associated with anti-AP-1 properties and led to development of a number of potent AP-1 inhibitors. In addition, some natural products bearing AP-1 inhibitory activity are also described herein. There appears to be no generally accepted system of classifying such molecules, and this review relies on broad structural similarity and seeks to provide an update on research advances of the past 2 decades in the development of chemical entities as AP-1 inhibitors.

5.1. SP100030 Analogues. SP100030 (**1**, Figure 4) is one of the first reported potent small molecule inhibitors of AP-1 and NF- κ B transcription activation with an IC₅₀ value of 0.05 μ M.¹⁰⁷ It is about 10-fold more potent than the earlier hit **2** (IC₅₀ = 0.5 μ M), which has been identified by using automated high-throughput assays with stably transfected human Jurkat T-cells.^{109,110} Hit **2** was reported to have an inhibitory effect on the production of IL-2 and IL-8 levels in stimulated cells, and it was found to be efficacious in an animal model of inflammation.¹⁰⁹ In order to increase the potency of hit **2**, hundreds of compounds have been designed and synthesized through the use of solution-phase parallel chemistry and targeted synthesis and **1** was identified as the most potent analogue. **1** has been demonstrated to selectively inhibit CD8(+) T-cells and mRNA expression of both Th1 and Th2 cytokines in vivo instead of inhibiting allergen-induced airway eosinophilia and bronchial hyperresponsiveness (BHR) in a rat model of asthma.⁵² In addition, **1** was confirmed active in a dose-dependent manner in several animal models of inflammation and immunosuppression (ip, 10–20 mg/kg).¹¹⁰ Moreover, daily subcutaneous injection of cachectic Yoshida

AH-130 ascites hepatoma-bearing rats with **1** at a dose of 1 mg/kg resulted in a clear amelioration of the cachectic effect, especially at the level of skeletal muscle.¹¹¹ At this dose, **1** acts as an effective inhibitor of AP-1, while the NF- κ B transcription factor is not affected, suggesting that the AP-1 signaling cascade plays an important role in the signaling of muscle wasting associated with disease.¹¹¹ However, the low aqueous solubility and high lipophilicity of **1** likely gave reason for the lack of poor oral activity in the animal models, consistent with its poor permeability in the gastrointestinal cell line, Caco-2 (apparent permeability coefficient P_{app} = $(11 \pm 4) \times 10^{-7}$ cm/s).¹¹²

To improve its potential oral bioavailability and Caco-2 permeability, a series of analogues of **1** modified at the 2, 4, 5, and 6 positions of the pyrimidine ring as shown in Figure 4 have been prepared by using a solution-phase parallel synthesis technique. All these compounds meet the criteria of Lipinski's "rule of five". The introduction of a fluoro group in the place of 2-chloro of **1** results in compound **3** with a comparable activity (IC₅₀ of 0.01 vs 0.05 μ M). However, other substitutions at the 2-position lead to a loss of activity, such as compounds **4** and **5** (Table 3). The trifluoromethyl group at the 4-position can be replaced with a methyl (**6**), chloro (**7**), or phenyl (**8**) with slight changes of activity. The carboxamide group at the 5-position is critical for activity. When it is moved to the 6-position (compounds **9** and **10**), the activity is totally lost. Among these, the 2-methyl analogue (**6**) shows comparable in vitro activity to **1** (IC₅₀ of 0.02 vs 0.05 μ M), with improved Caco-2 permeability (P_{app} of $(62 \pm 6) \times 10^{-7}$ vs $(11 \pm 4) \times 10^{-7}$ cm/s) and potential oral bioavailability.¹¹²

Palanki and co-workers¹¹² also investigated the importance of the pyrimidine by replacement with several other ring systems while retaining the *N*-(3',5'-bis(trifluoromethyl)-phenyl)carboxamide framework. Phenyl analogue **11** is completely inactive, while pyridazines (**12** and **13**) and pyrazine **14** are less potent than **1**.¹¹² Surprisingly, compound **13** with a 5-hydrogen is more potent than the 5-trifluoromethyl compound **12** (IC₅₀ of 1.7 vs 10 μ M),¹¹² which is somewhat

Table 3. Inhibition of SP100030 Analogues on AP-1 and NF- κ B Mediated Transcriptional Activation in Luciferase Reporter Assays on Jurkat T-Cells

compd	IC ₅₀ μ M	
	AP-1	NF- κ B
1	0.05	0.05
2	0.50	0.50
3	0.1	0.4
4	>10	>10
5	>10	>10
6	0.02	0.05
7	0.2	0.6
8	0.4	0.4
9	>10	>10
10	>10	>10
11	>30	>30
12	10	10
13	1.7	1.7
14	4.0	4.0
21	10	10
22	9.4	13
23	9.4	24
24	6.1	4.2
25	8.5	8.0
26	10	10
27	1.9	1.3
28	0.1	0.2

different from the compounds with a pyrimidine ring system. However, compound **12** is 34-fold less potent than **1** (IC₅₀ of 1.7 vs 0.05 μ M) and exhibits a comparable potency to its 1,4-isomer **14** (IC₅₀ of 1.7 vs 4 μ M).¹¹² In addition, replacing the 2-chloro-4-trifluorobenzamide in **11** with 2-halogen-4-hydroxybenzamide results in compounds **15–17**, which display good inhibitory effects on AP-1 mediated transcriptional activation in the order of Br > Cl > I (Table 4) and are more potent than their corresponding 3,5-dichlorophenyl analogues **18–20**.¹¹³

Table 4. Inhibition of AP-1 Mediated Transcriptional Activation in HeLa Cell Line Based in Vitro Assay

compd	inhibition, %	
	10 μ g/mL	1 μ g/mL
15	89.1	42.4
16	91.2	48.4
17	82.4	25.4
18	74.8	22.7
19	83.8	39.3
20	75.4	ND ^a

^aND: not determined.

On the other hand, in an effort to increase the druglike properties of highly lipophilic **1**, Palanki and co-workers¹¹⁴ have also designed several conformationally restricted analogues in which the pyrimidine ring and the aniline ring are connected through a second “bridge”. In fact, their original plan was only to simply substitute one or two trifluoromethyl groups in **1** with any other groups, but all initial attempts resulted in a loss of activity.¹¹⁵ As shown in Figure 5, compounds **21–23** by restricting one or two dihedral angles are about 200-fold less potent than **1** (Table 3). All three dihedral angles are restricted in compounds **24–28**. The direct bridged analogue **24**, the

carbon-bridged **25**, and the nitrogen-bridged **26** display weaker activity and are 120- to 200-fold less potent than **1**. However, the oxygen-bridged analogue **27** is 3- to 5-fold more potent than **24–26**. Interestingly, the potency of seven-membered sulfur-bridged analogue **28** bearing only one trifluoromethyl group is comparable to that of **1** (IC₅₀ of 0.1 vs 0.05 μ M), representing the most potent in this series. The seven-membered sulfur-bridged analogues thus provide a new class of inhibitors for AP-1 and NF- κ B mediated transcriptional activation.¹¹⁴

5.2. SPC-839 Analogues. By screening their diversified compound library using automated high-throughput assays with stably transfected human Jurkat T-cells, Palanki and colleagues^{116,117} have also identified another two novel hits, **29** and its isomer **39** (Figures 6 and 7), which display a slightly lower potency than their earlier hit **2** (IC₅₀ of 2.0 and 1.0 vs 0.5 μ M, respectively). Both hits **29**¹¹⁷ and **39**¹¹⁶ were found to inhibit AP-1 and NF- κ B mediated transcriptional activation without blocking basal transcription driven by the β -actin promoter and to exhibit a similar inhibitory effect on the production of IL-2 and IL-8 levels in stimulated Jurkat T-cells.

To improve the potency of **29**, different substituents around the 2, 4, and 5 positions of the pyrimidine ring were explored.¹¹⁷ As shown in Figure 6, all these compounds display similar IC₅₀ values in both AP-1 and NF- κ B assays. The removal of a methyl group or the introduction of a phenyl group in place of methyl group or the introduction of another methyl group on the citraconamido ring of the 2-position of the pyrimidine ring in **29** results in a slight change in activity (IC₅₀ of 1.6–3.9 vs 2.0 μ M, Table 5). However, removal of the citraconamido ring leads to a much weaker potency (IC₅₀ = 30 μ M). When the hydrogen of NH group at the 2-position is replaced with an alkyl, substituted carbonyl, urea, or carbamate group, all these compounds exhibit 2- to 6-fold improvement in potency (IC₅₀ of 0.3–0.83 vs 2.0 μ M). Among them, N-methylated 2-amino group of **29** as the most potent compound displays an improved potency in comparison with **30** (IC₅₀ of 0.3 vs 2.0 μ M). At the 4-position of the pyrimidine ring, replacement of the trifluoromethyl with a methyl group results in a compound with a comparable potency, while that with an ethyl (**31**) or pentafluoroethyl group leads to an improved potency (IC₅₀ of 0.2–0.4 vs 2.0 μ M). Moreover, replacement of the trifluoromethyl of **30** with an ethyl group can further improve its potency about another 8-fold (**32**, IC₅₀ of 0.035 vs 0.3 μ M). Substitutions with bulky groups such as phenyl and benzyl are not favorable to improve the potency in general, while 2-(5-methylthienyl) analogue (**33**) displays a better potency with 40-fold improvement with an IC₅₀ value of 0.045 μ M. The introduction of a bulkier alkyl ester group such as *tert*-butyl ester (**34**) instead of ethyl ester at the 5-position leads to about a 10-fold increase in potency (IC₅₀ of 0.21 vs 2.0 μ M). Other substitution groups such as a carboxylic acid, carboxamide, or *N,N*-dimethylcarboxamide result in a loss of potency (IC₅₀ = 30 μ M). Compounds **35** and **36** with a methyl ketone or a phenyl ketone at the 5-position exhibit an improved potency with an IC₅₀ value of 4.4 and 0.098 μ M, respectively. Several bioisosteres of ethyl ester such as oxazoline, isoxazole, oxadiazole, tetrazole, or phenyloxazole moieties were found to display a lower potency than **29** (IC₅₀ of 2.8–10 μ M), while analogues **37** and **38** with the methyloxazole exhibit a slightly increased potency.¹¹⁷

Upon the basis of hit **39** as the chemical lead, different substituents at the 2 and 4 positions of the pyrimidine ring have

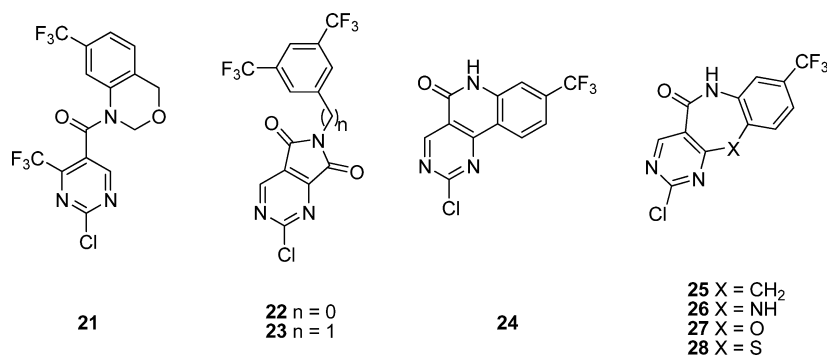


Figure 5. Chemical structures of 21–28.

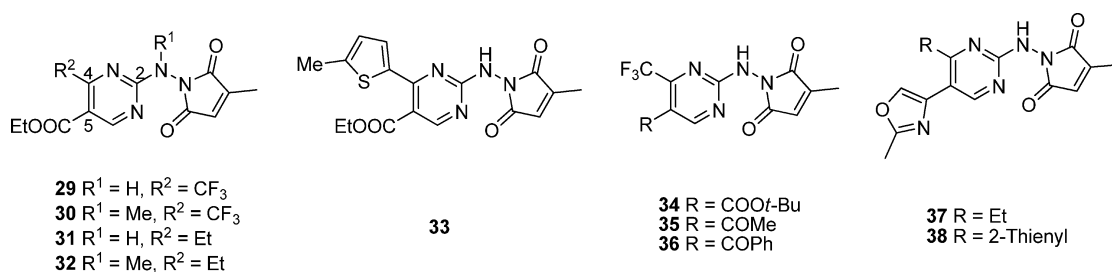


Figure 6. Chemical structures of analogues based on modifications of hit 29.

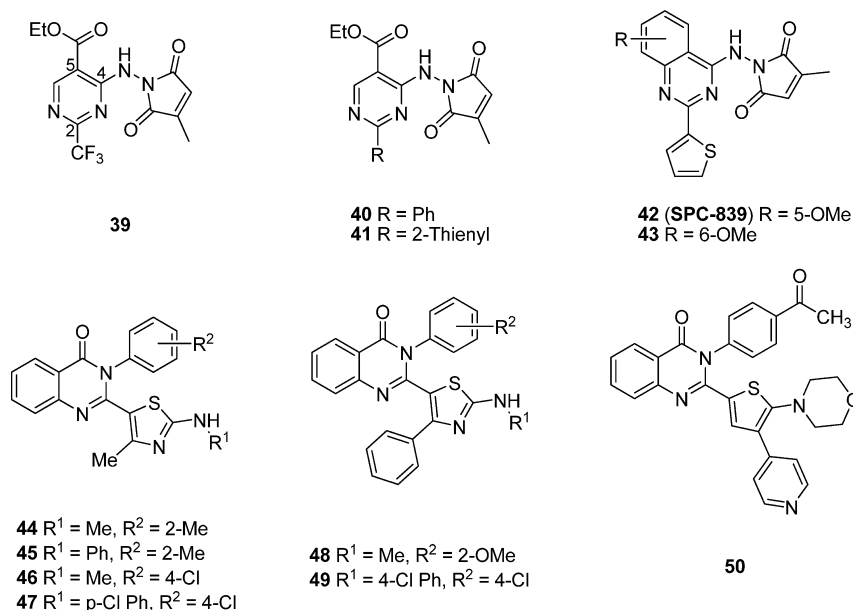


Figure 7. Chemical structures of analogues based on modifications of hit 39.

also been investigated to improve its potency.¹¹⁶ As shown in Figure 7, the substitutions with small alkyl groups such as methyl and ethyl instead of trifluoromethyl moiety at the 2-position generally result in a loss of activity, while bulkier groups such as *tert*-butyl group can retain the activity with a comparable potency. The substituted or unsubstituted heterocyclic rings at 2-position of the hit generally lead to a reduced activity. However, analogues 40 and 41 with a phenyl or 2-thienyl moiety exhibit a 10- to 50-fold improved activity (IC_{50} of 0.1–0.02 vs 1.0 μM , Table 4). Other substituents on the phenyl ring of 40 or the thienyl ring of 41 appear to be less favorable. The removal of a methyl group or the introduction of a phenyl group instead of methyl or the introduction of an

additional methyl group into the citraconamido ring, as well as methylation or acetylation of the NH group at the 4-position of the pyrimidine ring in 39 results in a decreased activity. Unfortunately, the most potent compound 41 was found to display no oral activity in rat PK studies and poor Caco-2 permeability likely due to the existence of the carboxylate moiety.¹¹⁸ In addition, several analogues of 41 with oxazoline, isoxazole, oxadiazole, or tetrazole as the carboxylate bioisosteres at the 5-position of pyrimidine ring turn out to be less potent.¹¹⁶

To improve the druglikeness of highly lipophilic lead 41, a new class of inhibitors without a carboxylate ester function have been designed and synthesized by introducing a fused phenyl

Table 5. Inhibition of SPC-839 Analogues on AP-1 and NF- κ B Mediated Transcriptional Activation in Luciferase Reporter Assays on Jurkat T-Cells

compd	IC ₅₀ , μ M	
	AP-1	NF- κ B
29	2	2
30	0.3	0.3
31	0.4	0.4
32	0.035	0.035
33	0.045	0.045
34	0.21	0.21
35	4.4	4.4
36	0.098	0.098
37	0.83	0.83
38	0.76	0.76
39	1	1
40	0.1	0.1
41	0.02	0.02
42	0.008	0.008
43	0.003	0.003

ring on the pyrimidine with a methoxy group at its 5-position (Figure 7).¹¹⁸ By changing the position and number of the methoxy groups on the phenyl of the quinazoline ring, as well as replacement with other electron-withdrawing or -donating groups, Palanki and co-workers¹¹⁸ have identified several improved compounds with a methoxy group at the 5-position (42) or 6-position (43) with 2.5- to 6.7-fold improvement compared to 41 (IC₅₀ of 0.008–0.003 vs 0.02 μ M). Compound 42 (SPC-839) displays higher permeability than 43 (P_c of $(1.41 \pm 0.4) \times 10^{-5}$ vs $(1.62 \pm 0.2) \times 10^{-6}$ cm/s). Moreover, 42 has also been demonstrated to be more efficacious in an adjuvant-induced arthritis rat model by reducing the swelling by 65% in the noninjected foot.¹¹⁸

On the basis of compound 42 as the advanced chemical lead and utilizing the bioisosterism and other medicinal chemistry optimization approaches, Giri and colleagues¹¹⁹ have designed and investigated a series of new compounds with the (2-(2,4-disubstituted-thiazole-5-yl)-3-aryl-3H-quinazolin-4-one scaffold as potential inhibitors of NF- κ B and/or AP-1 mediated transcriptional activation for developing anti-inflammatory agents (Figure 7). 44 (IC₅₀ = 5.5 μ M) and 45 (IC₅₀ = 5.5 μ M) have been identified to be more selective toward inhibiting AP-1 mediated transcriptional activity over NF- κ B in luciferase reporter assays on HEK293 cells. Compound 48 turns out to be the most potent dual inhibitors of NF- κ B and AP-1 mediated transcriptional activation with an IC₅₀ value of 0.2 and 0.5 μ M, respectively.¹²⁰ 48 has also been demonstrated to have significant in vivo efficacy in a carrageenan injection-induced

inflammation model with 56% inhibition of rat paw edema.¹²⁰ Additionally, Giri and co-workers¹²¹ have also evaluated the potential of the novel scaffold mentioned above for cancer by inhibiting multiple pathways in luciferase reporter assays on HEK293 cells. 46 and 47 (IC₅₀ of 1.2–3.0 μ M) are more selective toward inhibiting AP-1 mediated transcriptional activity, while 49 is a potent dual inhibitor of both NF- κ B and AP-1 mediated transcriptional activation with IC₅₀ values of 3.3 and 4.3 μ M, respectively. By replacement of the trisubstituted thiazole moiety with a thiophenyl ring, a series of 2-(2,3-disubstituted-thiophen-5-yl)-3H-quinazolin-4-one analogues have been designed and explored as potential anti-inflammatory and anticancer agents.¹²¹ Compound 50 is the most potent dual inhibitor of NF- κ B and AP-1 mediated transcriptional activation from the entire series, with IC₅₀ values of 10 and 5 μ M for NF- κ B and AP-1, respectively.

5.3. T-5224 Analogues. 51 (Figure 8), an inhibitor of the c-Fos/AP-1, has been identified by converting cyclic disulfide decapeptides¹²² to a series of nonpeptidic benzophenone derivatives¹²³ using a lead-hopping approach based on a 3D pharmacophore model. 51 specifically inhibits the DNA binding activity of c-Fos/c-Jun without affecting those of other transcription factors including C/EBP α and ATF-2 (bZIP domain), MyoD (basic helix–loop–helix domain), Sp-1 (zinc-finger domain), and NF- κ B/p65 (Rel homology domain), as well as the levels of c-Fos family protein members themselves.¹²⁴ Administration of 51 at a dose of 30 mg/kg was found to resolve type II collagen-induced arthritis (CIA) in a preclinical model by reducing the amount of inflammatory cytokines including interleukin 1 β and matrix-degrading MMPs in vivo in sera and joints as well as in vitro in synovial cell and chondrocyte cultures.¹²⁴ 51 also synergizes with antitumor necrosis factor α (TNF α), a signaling molecule immediately downstream of c-Fos, to inhibit arthritis.¹²⁴

Animal studies revealed that the major metabolites of 51 were glucuronides.⁵ In addition, glucuronides were also found to be major metabolites in human urine. By use of human liver microsomes (HLMs), human intestinal microsomes (HIMs), recombinant human cytochrome P450 (CYP450), and UDP-glucuronosyltransferases (UGTs) isoforms expressed in baculovirus-infected insect cells, it was predicted that 51 was converted to its acyl O-glucuronide by UGT1A1 and UGT1A3 and to its hydroxyl O-glucuronide by several UGTs, but it was not metabolized by the P450.⁵ Moreover, the glucuronidation of 51 was estimated to predominantly occur in the liver by comparing the intrinsic clearances (CL_{int}) between HLM and HIM.

On the basis of its promising pharmacological effects in arthritis, 51 has been developed by Toyama Chemical as a potential therapeutic agent for rheumatoid arthritis and has

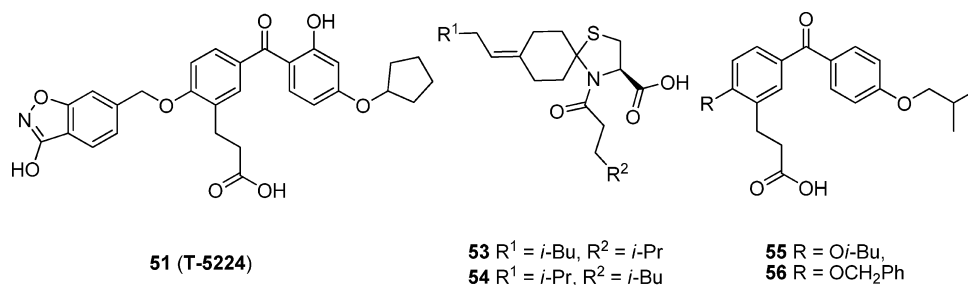


Figure 8. Discovery of 51, a selective AP-1 inhibitor in phase II human clinical trial.

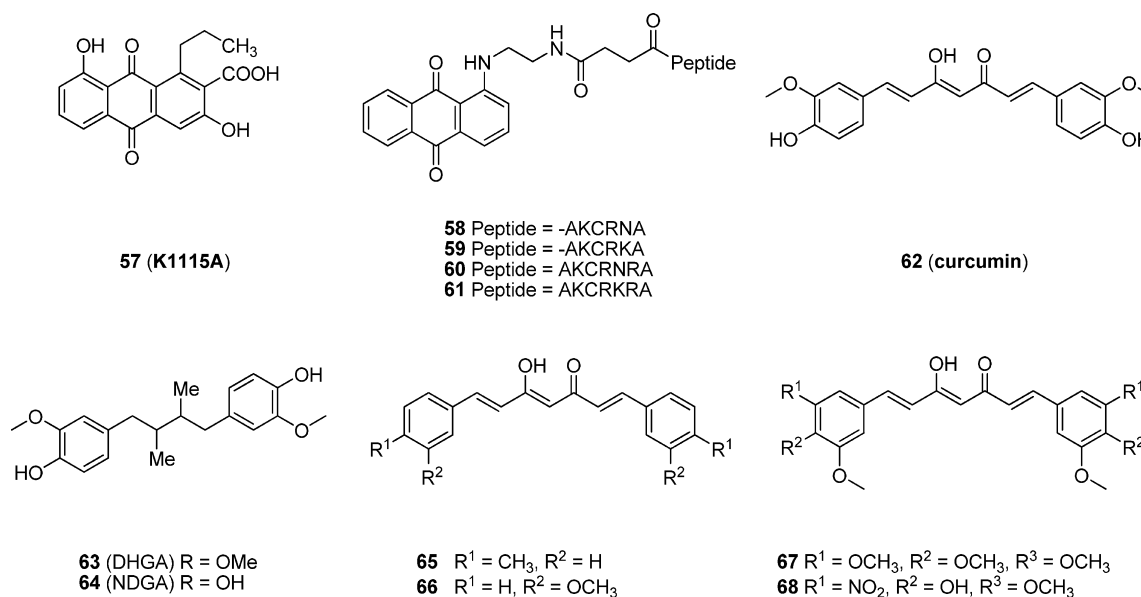


Figure 9. Chemical structures of K1115A, curcumin, and their analogues. Abbreviations in the peptides are the following: A, Ala; K, Lys; C, Cys; N, Asp; R, Arg.

advanced into human phase II clinical trials in Japan. Recently, it has also been reported that **51** not only can ameliorate lipopolysaccharide (LPS) induced liver injury through decreasing production of proinflammatory cytokines and chemokines in endotoxemic mice¹²⁵ but also has a potential inhibitory effect against endotoxin-induced acute kidney injury (AKI) by suppressing the TNF- α inflammatory response and other downstream effectors.¹²⁶

The cyclic disulfide decapeptides, especially the most potent inhibitor Ac-c[Cys-Gly-Gln-Leu-Asp-Leu-Ala-Asp-Gly-Cys]-NH₂ (**52**), have been designed and synthesized as inhibitors of the c-Fos/AP-1 de novo by three-dimensional pharmacophore modeling¹²² based on the X-ray crystal structure of the bZIP domain of the AP-1–DNA complex.⁸ A hypothetical 3D pharmacophore model was then constructed for generating new c-Fos/AP-1 inhibitors based on an alanine scan experiment, molecular dynamics simulation of the bZIP–**52** complex, and NMR measurement of the peptide in water.¹²² The pharmacophore consists of three hydrophobic groups, one hydrogen bond acceptor or donor, and one acidic group. Using a lead-hopping strategy based on the 3D pharmacophore model, Tsuchida and co-workers¹²³ discovered new non-peptidic small-molecule AP-1 inhibitors based on 1-thia-4-azaspiro[4.5]decane and benzophenone derivatives, which were synthetically accessible and easy to optimize.

As shown in Figure 8, compounds **53** and **54** bearing the scaffold of 1-thia-4-azaspiro[4.5]decane and compounds **55** and **56** bearing the scaffold of benzophenone display lower inhibitory activity than peptide **52** (IC₅₀ = 64 μ M) on the binding of AP-1 bZIP and oligonucleotides containing the AP-1 binding site (IC₅₀ of 420–650 μ M) using an ELISA-based AP-1 DNA-binding assay. Additionally, these compounds also inhibit the expression of AP-1-luciferase by TPA-stimulated NIH3T3 cell (IC₅₀ of 5.0–13.3 μ M). However, **51** can fit the cyclic peptide **52**-derived model well, with an IC₅₀ value of \sim 10 μ M for most of the in vitro cellular assays including luciferase assays. Moreover, in vivo ED₅₀ of **51** is \sim 1–10 mg/kg, and the C_{max} is 0.03–0.5 μ M (15–240 ng/mL).¹²⁴ Compound **51** is the

only selective AP-1 inhibitor that has been advanced into human clinical trials to date.

5.4. Natural Products and Other Compounds. K1115 A (**57**, Figure 9), a new anthraquinone derivative, was isolated from the culture broth of *Streptomyces griseorubiginosus* (Mer-K1115).¹²⁷ **57** inhibits the direct binding of AP-1 to AP-1 oligonucleotide (IC₅₀ = 100 μ M) and the production of collagenase in IL-1 α -stimulated rat synovial cells (IC₅₀ = 60 μ M) in vitro. Moreover, **57** can attenuate the inflammatory response mediated by AP-1 through decreasing the ornithine decarboxylase (ODC) activity of phorbol myristate acetate (PMA) induced mice. Given that certain truncated peptides would have the potential to bind the DNA AP-1 consensus sequence under the same conditions as the native AP-1 protein, Patterson and co-workers¹²⁸ have designed a series of peptide–anthraquinone conjugates as inhibitors of AP-1 transcription factor. Truncated AP-1-like peptides, with five to seven residues, bearing a highly conserved sequence motif lysine–cysteine–arginine (KCR) were attached at the N-terminus to an intercalating anthraquinone moiety of **57** through an amino acid type linker. All these anthraquinone–peptides were found to displace AP-1 protein binding from its DNA consensus sequence much more effectively than their respective free peptides using the electrophoretic mobility shift assay (EMSA). Moreover, the peptide conjugates containing the more basic sequences 2-AKCRNA (**58**), 2-AKCRKA (**59**), 2-AKCRNRA (**60**), and 2-AKCRKRA (**61**) were demonstrated to be the most effective inhibitors in this series.¹²⁸

Curcumin (**62**, Figure 9), a hydrophobic polyphenol derived from *Curcuma longa*, a plant of the ginger family, is well-known to have diverse biological functions such as anti-inflammatory, antitumor, antioxidative, cytotoxic, antifungal, antibacterial, and antihepatotoxic activities.¹²⁹ Curcumin was reported to inhibit TPA-induced expression of c-Fos and c-Jun protooncogene mRNA and to repress c-Jun binding to its cognate motif in NIH3T3 mouse fibroblast cells.^{130,131} Additionally, suppression of AP-1 binding to DNA was also observed in human leukemia cells¹³² and transformed keratinocytes.¹³³ Furthermore, curcumin displays an inhibitory effect against the complex formation

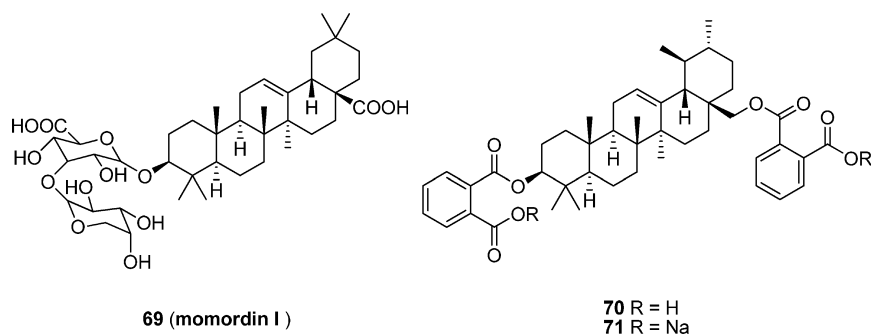


Figure 10. Momordin I and its analogues.

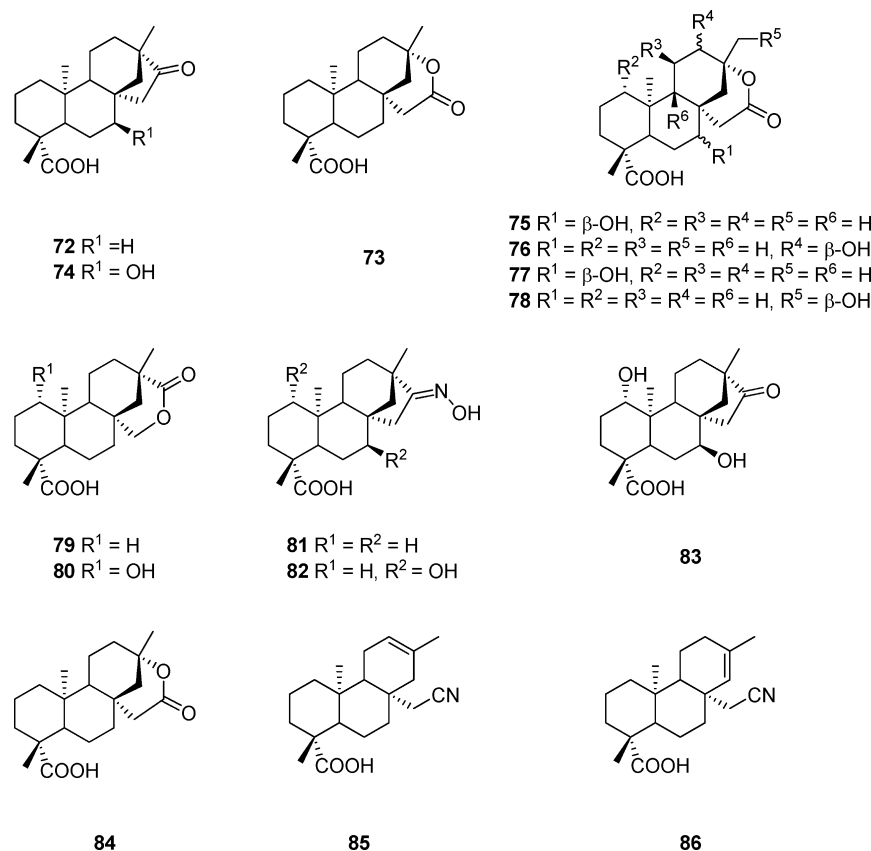


Figure 11. Isosteviol and its analogues.

of the Fos–Jun dimer and the DNA consensus sequence with an IC₅₀ value of 6.9 nM and is about 30-fold more potent than dihydroguaiaretic acid (DHGA, **63**), isolated from the aryls of *Myristicafragrans* (IC₅₀ = 0.21 μ M).¹³⁴ However, the analogue of DHGA, nordihydroguaiaretic acid (NDGA, **64**), exhibits a potency comparable to that of curcumin (IC₅₀ = 7.9 nM). Both DHGA and NDGA are capable of suppressing leukemia and lung and colon cancer in the MTT-based bioassay.¹³⁴ Curcumin has also progressed to human clinical trials, but it is a nonselective AP-1 inhibitor mixed with activities of other targets including various transcription factors, thereby significantly limiting its potential as a targeted therapy.

To improve the potency and selectivity of curcumin-based compounds, Yang and colleagues¹³⁵ synthesized a series of symmetrical curcumin analogues and evaluated their inhibitory activity on Fos–Jun complex formation through the EMSA experiment. As shown in Figure 9, curcuminoids **65** and **66** exhibit an IC₅₀ value of 8.98 and 5.40 μ M, respectively, which

are 60- to 100-fold more potent than curcumin (IC₅₀ = 540 μ M). In addition, through inhibition of AP-1 transcription, thereby down-regulating the expression of angiogenesis-associated genes (VEGF and MMP-9), curcumin analogues **67** and **68** were found to show the angiogenesis inhibitory effect on the developmental neovascularization of chicken embryonic with 68–88% inhibition of embryos exposed to 10 μ g of the compound treatment.¹³⁶ Their inhibitory effects on angiogenesis have also been confirmed by wound migration, invasion, and tube formation assays.¹³⁶

Momordin I (**69**), isolated from *Ampelopsis radix*, and its disaccharide and carboxylic acid modified derivatives **70** and **71** (Figure 10) have demonstrated a remarkable inhibitory effect on the formation of Fos–Jun DNA complex.¹³⁷ Momordin I derivatives **70** and **71** display an IC₅₀ value of approximately 4.0 μ M in EMSA assay, which is 30-fold more potent than momordin I (IC₅₀ = 0.13 mM) and about 125-fold more effective than curcumin (IC₅₀ = 0.48 mM).

Microbial transformation is an area of great interest for applying biocatalysis to selectively convert synthetic and natural products to biological compounds that are difficult to obtain by conventional chemical methods.^{138,139} Special attention has been paid to filamentous fungi because of their capability of catalyzing regio- and stereoselective hydroxylation of a variety of nonfunctionalized hydrocarbon centers of a great variety of substrates.¹⁴⁰ Isosteviol (**72**, Figure 11), with a rigid skeleton comprising four fused rings similar to the steroid skeleton, possesses various biological activities.¹⁴¹ Isosteviol lactone (**73**), an *ent*-beyerane tetracyclic diterpenoid prepared by reacting isosteviol (**72**) with *m*-chloroperbenzoic acid, was investigated for its activity on mitochondrial metabolism.¹⁴² A number of hydroxylated diterpenoids have been generated from the microbial transformation of isosteviol lactone (**73**) with *Mucorrecurvatus* MR 36, *Aspergillusniger* BCRC 31130, or *Absidiapseudocylindrospora* ATCC 24169. As shown in Table 6, compounds **73**–**80** exhibit significant inhibitory effects on

Table 6. Luciferase Activity of Isosteviol Analogues^a

compd	luciferase activity	compd	luciferase activity
73	2.38 ± 0.18	81	4.87 ± 2.15
74	2.69 ± 0.92	82	2.69 ± 0.45
75	2.39 ± 0.64	83	2.98 ± 0.35
76	2.73 ± 0.58	84	2.38 ± 0.18
77	3.02 ± 0.71	85	2.22 ± 0.35
78	2.58 ± 0.86	86	2.85 ± 0.48
79	1.88 ± 0.39	control	3.95 ± 0.53
80	2.66 ± 0.99	dexamethasone	2.33 ± 0.36

^aThe concentration of each test compound was 10 μ M. All luciferase activities were normalized to Renilla luciferase activity. The data were expressed as multiples of luciferase activity compared to the no-treatment (control) group. Dexamethasone is the reference compound.

AP-1 activation in lipopolysaccharide-stimulated RAW 264.7 macrophages by AP-1-mediated luciferase reporter gene assay, while **79** is more potent than the reference compound of dexamethasone.¹⁴² Given that the important biological properties of steroids are dependent upon its D-ring, a series of compounds with modified D ring have been produced by microbial transformation of isosteviol oxime (**81**) with *Aspergillus niger* BCRC 32720 and *Absidia pseudocylindrospora* ATCC 24169.¹⁴³ Among the compounds tested, **82**–**86** significantly inhibit AP-1 activation in LPS-stimulated RAW 264.7 macrophages, and in particular, **85** displays an inhibitory activity more potent than dexamethasone.¹⁴³

Other than diterpenes, norditerpenes and triterpenes can also inhibit the AP-1 activity. As shown in Figure 12, norditerpenes nagilactone (**87**) and inumakilactone (**88**),¹⁴⁴ isolated from an organic solvent extract of the root bark of *Podocarpus latifolius* (Thunb.) collected in Tanzania, have also been demonstrated to be capable of inhibiting phorbol ester TPA-induced activation of AP-1 activity at the concentrations tested, with IC_{50} values estimated from dose–response curves of 1.5 and 4.0 μ M, respectively. However, both of them also appear to be toxic, with cell survival of <50% in each case at more than 2.5 μ M.¹⁴⁴ Quassinoids glaucarubinone (**89**) and nothospondin (**90**) also display potent, dose-dependent AP-1 inhibition at noncytotoxic concentrations by a β -lactamase driven reporter assay using fluorescence resonance energy transfer (FRET) technology, with EC_{50} values of 1.49 and 0.13 μ M,

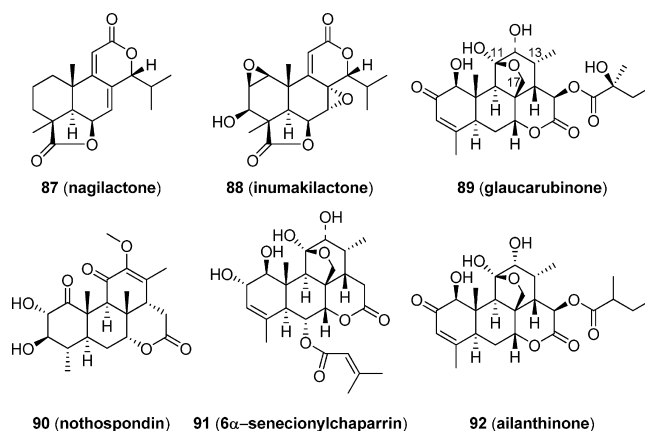


Figure 12. Chemical structures of quassinoids with AP-1 inhibitory effects.

respectively.¹⁴⁵ The potent AP-1 inhibitory activity of **89** may be ascribed to its ether bridge between C-17 and C-11, which is seen in many other AP-1 active quassinoids, such as 6 α -senecionylchaparrin (**91**) and ailanthinone (**92**).¹⁴⁶ However, **90** is the first quassinoid without an ether linkage that can inhibit AP-1, albeit at a significantly reduced potency.¹⁴⁵ In addition, **89** is noncytotoxic at a high concentration of 80 μ M by an XTT assay, while **90** shows some cytotoxicity with an IC_{50} of approximately 10 μ M.¹⁴⁵

Citrifolinin A (**93**, Figure 13), a new unusual iridoid isolated from the leaves of *Morindacitrifolia*, shows significant inhibition of UVB-induced AP-1 activity in cell cultures, with an IC_{50} of 69.6 μ M.¹⁴⁷ Citrifolinoside (**94**)¹⁴⁸ is another iridoid isolated from the leaves of *Morindacitrifolia* and displays a significant inhibitory effect on UVB-induced AP-1 activity and is 2.4-fold more potent than **93** (IC_{50} = 29.0 μ M).

From an extract of the Palauan cyanobacterium *Lyngbya majuscula*, grassypeptolides F (**95**, Figure 13) and G (**96**), bis-thiazoline-containing cyclic depsipeptides with a rare β -amino acid, extensive N-methylation, and a large number of D-amino acids, were isolated and found to have moderate inhibitory activity against the transcription factor AP-1 (IC_{50} of 5.2 and 6.0 μ M, respectively) in HEK293T cells.¹⁴⁹

In addition, compound (–)-**97**, a cycloadduct synthesized by tandem retro-Diels–Alder/Diels–Alder cycloadditions of *o*-quinol dimer under microwave irradiation, shows selective inhibition against AP-1 at 4 μ M in luciferase reporter assays on HEK293 cells (Figure 13).¹⁵⁰ Moreover, (–)-**97** does not display inhibition of HIF-2, NF- κ B, or SRE dependent transcription at the same concentration. However, during the experiments in the NCI 60-cell screen at 10 μ M, (–)-**97** did not pass the threshold for further evaluation of cell growth inhibition.

Retinoic acid is effective for chemotherapy and chemoprevention of cancer mediated either by blocking AP-1 activity or by activating retinoic acid response element (RARE).¹⁵¹ Using an AP-1-luciferase transgenic mouse as a carcinogenesis, Dong and colleagues¹⁵² provided the first in vivo evidence that **98** (SR11302), an AP-1 inhibition-specific retinoid, markedly inhibited both 12-*O*-tetradecanoylphorbol-13-acetate-induced papilloma formation and AP-1 activation in 7,12-dimethylbenzanthracene-initiated mouse skin. Interestingly, **98** does not activate transcription from the RARE and displays no activity at retinoic acid receptors (EC_{50} > 1 μ M for RAR α , RAR β , RAR γ , and RXR α).¹⁵² As an AP-1 inhibitor that displays antitumor

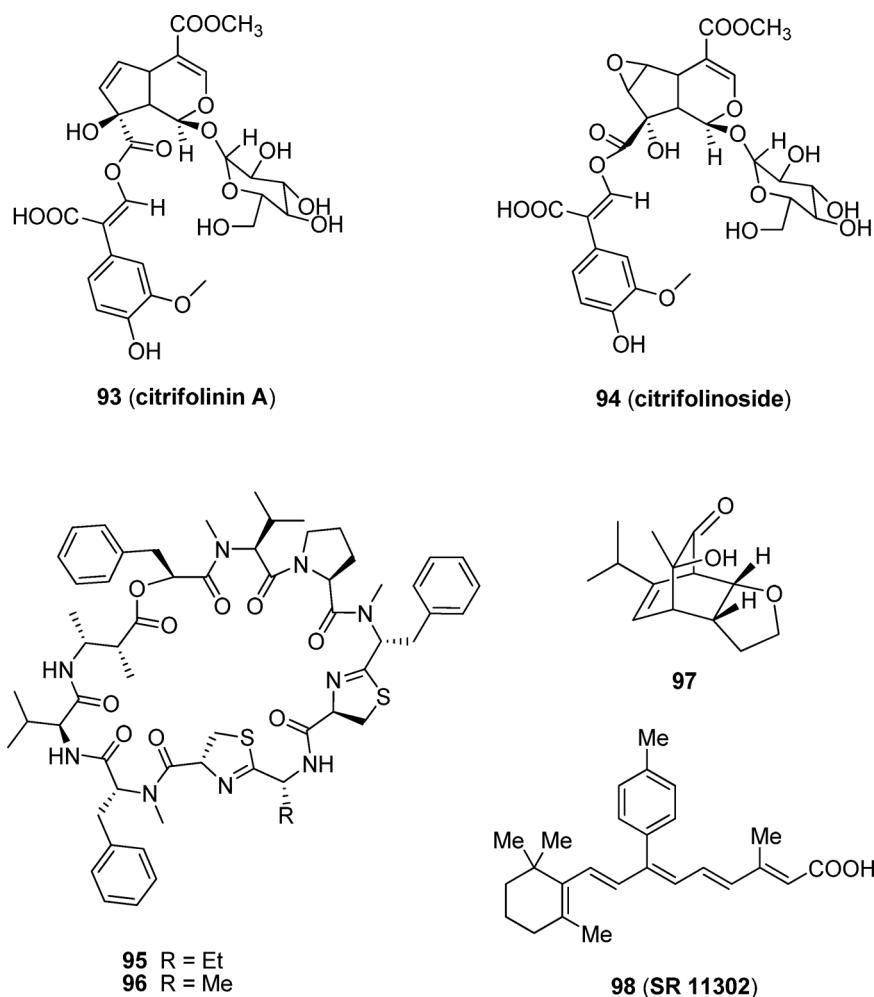


Figure 13. Natural products 93–96 and other AP-1 inhibitors 97 and 98.

effects in vivo, **98** serves as an important research tool compound that is commercially available from Tocris. In addition, some other nonspecific AP-1 inhibitors such as 6-shogaol and 6-gingerol have also been reported to inhibit 12-O-tetradecanoylphorbol-13-acetate-induced tumor promotion in mice.¹⁵³

6. CONCLUSIONS AND FUTURE DIRECTIONS

Since the discovery of AP-1 as the pivotal transcription factor in 1987,¹⁵⁴ significant progress has been made by many pioneers in understanding AP-1 biology and function and identifying AP-1 as an important and a valid therapeutic target for various human diseases. There is a great potential for the development of AP-1 inhibitors as preventive or therapeutic agents for cancer and inflammation, as well as a variety of other human conditions including hepatitis, pulmonary fibrosis, atherosclerosis, cardiovascular diseases, and Parkinson's disease. Although currently available drugs are helpful in alleviating many of the symptoms of these diseases, more targeted therapeutic approaches based upon the underlying mechanisms remain an urgent need for better specificity of the treatment with lower side effects. To this end, targeting AP-1 with potent and specific inhibitors may represent an attractive molecular approach for new medications.

The proof of concept by developing AP-1 inhibitors as a novel therapy for inflammation has been confirmed by the

investigation of clinical drug candidate **51**, although its fate is dependent on the outcomes of phase II human clinical trials. Other inhibitors, such as analogues of **1** and **42**, also have the potential to be developed as novel therapeutic agents. Nevertheless, researchers have spent tremendous efforts on hit-to-lead optimizations improving their druglike properties of the hits from high-throughput screening, especially their DMPK properties in vivo. Thus, safety concerns and in vivo pharmacokinetic alerts should be considered early in the drug discovery process. Fragment-based drug design (FBDD), by taking advantage of privileged fragments, may also provide an efficient approach to identify more effective and selective AP-1 inhibitors with better druglikeness.^{155–157}

On the other hand, it is worth noting that AP-1 may act as a double-edged sword in tumor development. By regulating genes involved in cell proliferation, differentiation, apoptosis, angiogenesis, and tumor invasion, AP-1 not only can be oncogenic but also can be antioncogenic.^{18,158} AP-1 activity in cancer seems to depend upon AP-1 dimer composition and tumor type as well as its differentiation state, tumor stage, and the genetic background of tumor.¹⁸ Generally, c-Jun mainly has oncogenic functions, while JunB and JunD have antioncogenic effects. As such, it would be quite challenging for medicinal chemists to design potent and specific AP-1 inhibitors as potential therapy for cancer. Individualized treatment may provide a solution to this problem by selecting appropriate

patient populations. Furthermore, if the antioncogenic function of some AP-1 proteins is properly exploited, it may provide a novel, unexpected, and more tractable direction for the discovery of anticancer treatments that can induce the formation of specific dimers for the selective and efficient killing of cancer cells, thereby actively interfering or antagonizing tumor development.

Despite the advances in high-throughput screening, structure, and ligand-based molecular modeling and hit-to-lead optimizations, which have identified numerous compounds capable of inhibiting AP-1 transcription activation or its DNA binding activity, only one selective AP-1 inhibitor **51** has progressed to human clinical trials. Meanwhile, it has to be pointed out that many of the currently available compounds display polypharmacological profiles by targeting various other transcription factors including NF- κ B. It is the opinion of the authors that because of the combinatorial diversity and expressed dependence of AP-1 family proteins, the multidisciplinary approaches with combined techniques in molecular pharmacology, chemical biology, and structural biology based on X-ray crystallography of protein–inhibitor complexes, as well as computational chemistry, will provide new insights into rational drug design and facilitate the search of novel AP-1 family selective inhibitors. Although those identified inhibitors of dual or multiple transcription factors may also be of value for treatment or prevention of diseases, discovery and development efforts remain an urgent need for medicinal chemists to yield more potent, efficacious, and specific AP-1 inhibitors as a viable therapeutic strategy for use in the human clinic.

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ABBREVIATIONS USED

CIA, collagen-induced arthritis; ATF, activating transcription factor; MAF, musculoaponeurotic fibrosarcoma; TPA, phorbol 12-*O*-tetradecanoate-13-acetate; NF- κ B, nuclear factor κ light chain enhancer of activated B cells; MAPK, mitogen-activated protein kinase; JNK, JUN amino terminal kinase; ES, embryonic stem; ERK, extracellular-signal-regulated kinase; HLM, human liver microsome; HIM, human intestinal microsome; PD, Parkinson's disease; TCF, ternary-complex factor; MEF2C, myocyte-enhancer factor 2C; LPS, lipopolysaccharide; AKI, acute kidney injury; CKII, casein kinase II; GSK-3 β , glycogen synthase kinase 3 β ; RSK2, ribosomal S6 kinase 2; NFAT, nuclear factor of activated T cells; STAT, signal transducer and activator of transcription; VEGF, vascular endothelial growth factor; MMP9, matrix metalloproteinase 9; MPP⁺, 1-methyl-4-phenylpyridinium ion; IC₅₀, 50% inhibitory concentration; ED₅₀, 50% effective dose; C_{max}, maximum

plasma concentration; SRE, serum response element; RARE, retinoic acid response element; RAR, retinoic acid receptor; CYP450, cytochrome P450; CL_{int} , intrinsic clearance; UGT, UDP-glucuronosyltransferase; FBDD, fragment-based drug design; DMPK, drug metabolism and pharmacokinetics

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