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Design and Synthesis of Orally Active Inhibitors of TNF Synthesis as Anti-rheumatoid Arthritis Drugs

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Abstract—A novel series of TNF inhibitors was identified based on the screening of existing MMP inhibitor libraries. Further SAR optimization led to the discovery of a novel lead compound. Its synthesis, efficacy in experimental animal models, and pharmacokinetic data are discussed.

Rheumatoid arthritis (RA)[1] is an autoimmune disease of unknown etiology characterized by chronic inflammation of symmetric, peripheral joints and, in some cases, by progressive joint erosions. The proinflammatory cytokine, tumor necrosis factor-α (TNF) is a key mediator of inflammatory processes in RA and in animal models of this disease.[2] TNF upregulates the expression of enzymes called matrix metalloproteinases (MMPs). Increased levels of several of these MMPs, including collagenase-3, stromelysin-1, and gelatinases A and B, have been implicated in joint destruction.[3]

The approval and subsequent commercial success of biologics such as TNF neutralizing antibodies and soluble receptors (Remicade® and Enbrel®) for the treatment of RA and Crohn's disease have validated the therapeutic effectiveness of TNF inhibition.[4] Although the current biological agents have ameliorated signs and symptoms, the delivery of these agents is parenteral and therapy is complicated by the development of serological responses against the biological therapies.[5] An orally administered agent that lowers endogenous TNF and has the same efficacy as biological TNF antagonists in rheumatoid patients would be an attractive drug for development. An inhibitor of TNF convertase could potentially accomplish this goal.[6,7]

TNF convertase (TACE) is a metalloproteinase that releases soluble TNF from the cell surface of macrophages. The enzyme cleaves the 26 KD membrane-bound form of TNF, thereby releasing soluble 17 KD TNF. TACE is a member of the adamalysin/ADAM subfamily of the metzincin superfamily, which also includes astacins, serralysins and MMPs.[8,9] Several non-selective MMP inhibitors were first shown to inhibit TNF release.[10,11] In the last few years a large number of TACE inhibitors, mostly succinimide, sulfonamide and phosphonamide based, have been published.[12] Here, we would like to disclose our orally bioavailable sulfamide based TACE inhibitors.[13]

Our starting point for the TACE project was the screening of our existing MMP libraries. Our primary screening assay used the inhibition of TNF release from the human monocytic cell line, Monomac6 (MM6), stimulated with lipopolysaccharide (LPS) and phorbol myristate acetate (PMA).[11] A number of leads were identified from different chemical classes but most of them were rejected for lead optimization due to difficult synthesis and/or poor pharmacokinetic properties. Compound **1** (Fig. 1), a relatively weak inhibitor of TNF release (IC_{50}, 5.8 μM), was chosen for further optimization based on the simplicity of the core structure. It was quickly learned that introduction of a double bond in the piperidine ring improved the potency by a factor of 5 (see **2**, MM6: 1.24 μM). A further improvement in the potency was observed when the

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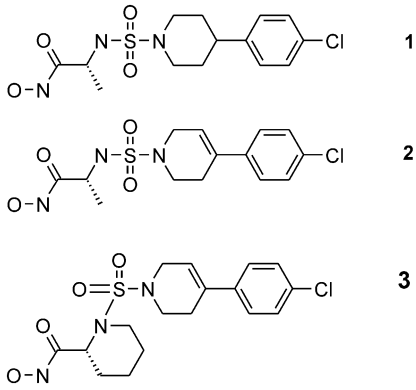


Figure 1. Lead identification.

amino acid was changed from alanine to pipecolic acid (see **3**, MM6: 430 nM).

We then looked at the effect of substitution on the phenyl ring, see Table 1. It is clear that large groups such as Ph at the *para* or *meta* positions of the phenyl ring are not tolerated. Relatively speaking the *meta* position will accommodate groups such as methoxy and ethyl better than the *para* position. But overall, the *p*-F or the unsubstituted analogues were the most potent compounds in the MM6 assay. Since the fluoro group at the *para* position might be expected to reduce the potential metabolic oxidation of the phenyl ring, we chose the *p*-F as the optimal substituent on the phenyl ring.

Several compounds from Table 1 were tested in the in vivo rat TNF inhibition assay. The inhibitors were dosed orally 30 min before LPS was injected intravenously. Blood was collected 90 min after LPS injection. TNF was measured in serum by ELISA. Only compound **5** gave $>50\%$ of inhibition when dosed orally at 60 mg/kg. PK for compound **5** in rat and dog indicated that the compound was cleared rapidly, especially in dog, when dosed orally (see discussion under Pharmacokinetics section).

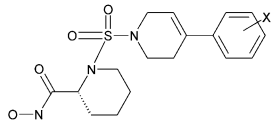
We speculated that the rapid clearance of the drug might be caused by the metabolic oxidation of the piperidine ring. We reasoned that we might be able to modulate the clearance by the introduction of a polar group on the piperidine ring. Replacement of the pipecolic acid by piperazic acid provides a site for such modification. Table 2 lists some of the SAR results obtained.

From the results in Table 2 it is clear that various substituents are tolerated on the nitrogen. The parent compound (**16**) is a much weaker inhibitor of TNF release although alkylation with trifluoroethyl restored the potency. The compounds were further screened in the rat LPS-induced TNF inhibition assays. Rat PK data were obtained to aid the compound selection. In the PK study the compounds were administrated orally at 30 mg/kg in 90% propylene glycol/10% ethanol to male Sprague–Dawley rats. Blood samples were collected at different time points and analyzed by HPLC. The AUC data (area under the concentration vs time curve calculated by the linear trapezoid rule) are listed in Table 2. Base on the MM6 and AUC datad, compound **17** was selected for further evaluation.

Murine Collagen-Induced Arthritis (MCIA)

Compound **17** was tested in the MCIA assay, a chronic model of RA.[14] In two separate experiments, partial reduction in the arthritic index for compound **17**-treated animals (dose 2–120 mg/kg po) was evident beginning 1 day after the start of dosing and persisting throughout the 14-day treatment period (Table 3). The reduction in arthritic index was 49–96% with a trend toward dose dependence. Statistically significant reductions in the arthritic index were achieved. The incidence, obtained by dividing the number of paws that were inflamed by the total number of paws at risk, was reduced significantly at all doses in both experiments. Compound **17** at 120 mg/kg also significantly reduced severity, quantitated by averaging the maximum scores of the

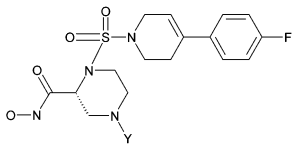
Table 1. The effect of substituent on the phenyl ring



Compd	X	MM6 (IC_{50}, μM[a])
4	H	0.24
5	*p*-F	0.3
6	*p*-Cl	0.48
7	*p*-Br	1.2
8	*p*-OCH_3	15
9	*m*-OCH_3	0.26
10	*p*-Ph	19
11	*m*-Ph	7.6
12	*p*-Oph	3.3
13	*m*,*p*-F_2	1.2
14	*m*-Et	0.32
15	*p*-$CH_2CH_2CH_3$	2.6

[a]Values are means of three experiments.

Table 2. The effect of substituents on the piperazine ring



Compd	Y	MM6 (IC_{50}, nM[a])	Rat PK, AUC (ng h/mL[b])
16	H	3560	na
17	$CONMe_2$	200	6180
18	CONHMe	120	1790
20	COmorpholino	350	7940
21	SO_2NMe_2	250	3270
22	$PO(NMe_2)_2$	510	1540
23	CH_2CF_3	300	3260

[a]Values are means of three experiments.
[b]Area under the concentration versus time curve calculated by the linear trapezoid rule when dosed 30 mg/kg orally. The detection limit for drug concentration was 50 ng/mL by HPLC/UV method.

Table 3. Compound **17** inhibits MCIA

Dose (mg/kg qd po)	Max. score (mean ± SD)	Inhibition (%)	Positive paws day 13 (%)	Severity (mean)
Vehicle	8.7 (±5.1)		70	3.2
2	2.9 (±4.1)	67*	27**	2.7
5	2.7 (±4.1)	69*	30**	2.4*
15	4.5 (±4.0)	49	41**	2.8
45	3.8 (±4.6)	56	33**	2.9
120	0.3 (±0.8)	96**	4**	1.0**

*$0.01 < p < 0.05$; ** $p < 0.01$.
Results from a single experiment are shown. Comparable results were obtained in a separate experiment.

inflamed paws. Additionally, following administration of 120 mg/kg orally complete protection of stifle, hock, and interphalangeal joints were observed histologically.

Pharmacokinetics

As mentioned earlier, the objective of introducing a polar functional group in **5** was to reduce its metabolic clearance. Table 4 lists the comparison of plasma concentrations for compound **5** and **17** in rat and dog. It is clear that the clearance of **17** is indeed much slower than that of compound **5**.

Syntheses

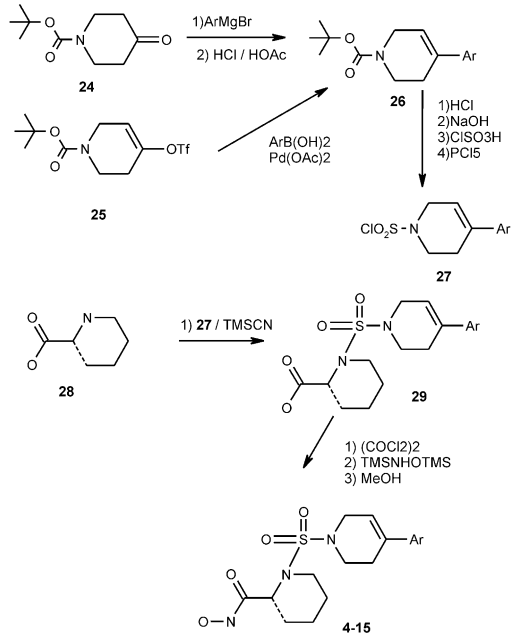
Compounds mentioned in this paper were synthesized according to the reactions shown in Schemes 1 and 2. The key step in Scheme 1 was the direct coupling of the sulfamoyl chloride **27** with the acid **28** which had been silylated with TMSCN first. The acid **29** was then converted to the acid chloride and coupled with bis-(trimethylsilyl)hydroxamine to give the final hydroxamate after aqueous workup. In Scheme 2, the acid **30** was first persilylated with HMDS and reacted sequentially with BocON and the sulfamoyl chloride **27**. The intermediate **32** underwent reaction with the appropriate electrophiles to give **33**.

Scheme 3 shows the process route for large-scale (kg) preparation of compound **17**. It was made from the acid **30** in two steps. The key step involves the dissolution of the dihydrochloride salt of acid **30** in HMDS and coupling the persilylated **30** sequentially with dimethylcarbamoyl chloride and **27** to produce acid **34** in 62% isolated yield. Acid **34** is converted to hydroxamic acid **17** via the acid chloride. The overall yield is 52%.

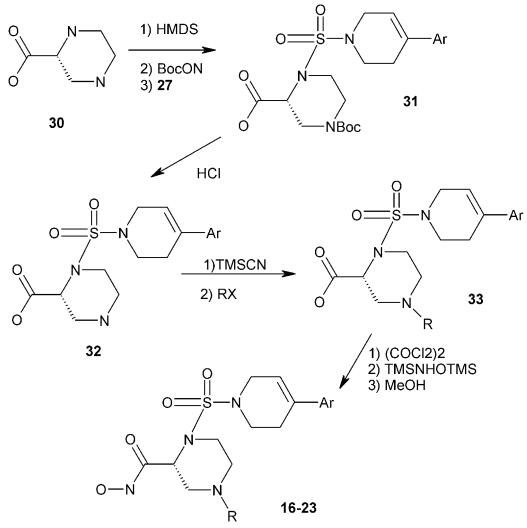
Table 4. Comparison of the PK parameters for compound **5** and **17**[a]

Concn (ng/mL)	**5**[b]	**17**
Rat Cmax	3060	1050
Rat C (@ 7 h)	0	233
Dog Cmax	117	182
Dog C (@ 2 h)	0	110

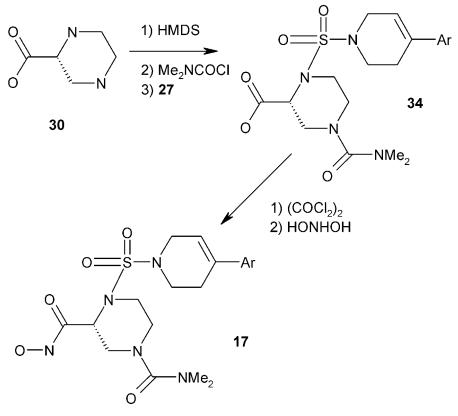
[a]Dosed orally at 30 mg/kg. The detection limit for the drug was 50 ng/mL using HPLC/UV method. The standard deviation for the HPLC assay was 20% and among animals was 40–50%.
[b]Racemic material was used.



Scheme 1. Preparation of compounds **4–15**.



Scheme 2. Preparation of compounds **16–23**.



Scheme 3. Preparation of compound **17**.

Summary

From the initial weak TNF inhibitor lead (5.8 μM) we were able to identify a series of potent and orally active TNF inhibitors. Compound **17** has been shown to inhibit the release of TNF in cells and in animals. It is active in a chronic RA model (MCIA) when administrated orally. The compound was advanced for further preclinical evaluation.

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