Phenylpyazoleanilides as Potent Inhibitor of IL-15 Dependent T Cell Proliferation. Part 2: Discovery of a New Drug Candidate, Y-320

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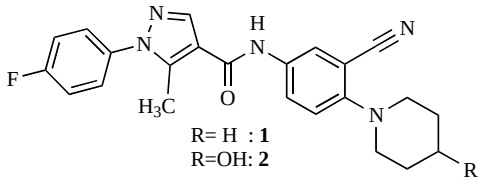
Abstract: Through the optimization of the active metabolite **2** of the piperidine derivative **1**, we discovered a potent new drug candidate for the treatment of RA, **Y-320** that inhibits T cell activation induced by IL-15 and shows a therapeutic effect on collagen-induced arthritis in DBA/1J mice. Design and structure-activity relationships are described.

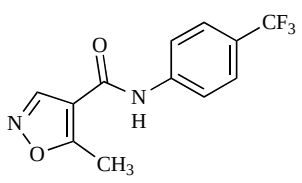
Keywords: Y-320, IL-15, Phenylpyrazoleanilide, RA, CIA.

INTRODUCTION

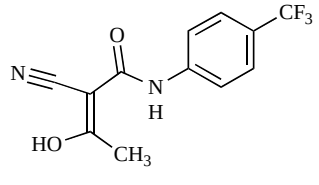
Rheumatoid arthritis (RA) is an autoimmune disorder characterized by synovial inflammation and the erosion of bone and cartilage, ultimately leading to the destruction of the affected joints [1-3]. A proinflammatory cytokine, interleukin 15 (IL-15) with T cell growth promoting activity was identified to play an important role in the pathgenesis and disease progression of RA. McInnes *et al.* reported that overexpression of IL-15 was observed in patients with active RA [4, 5]. They also suggested that IL-15 acts as a chemotactic and proliferation-inducing factor for T cells in RA joints [4, 5]. Moreover, the activities of IL-15 in RA are not limited to T cells but also extended to synoviocytes, macrophages, and osteoblasts because IL-15 can induce production of another proinflammatory cytokine, IL-17 by T cells [6, 7]. IL-17 works synoviocytes, macrophages, and osteoblasts to induce several inflammatory mediators including IL-6, IL-8, tumor necrosis factor-α, CC-chemokine ligand 2, matrix metalloproteinases, prostaglandin E_2, and receptor activator of NF-κB ligand [6, 7]. Consequently, IL-17 as well as IL-15 appears to play a critical role in the pathogenesis of RA.

These findings suggest that an inhibitor of IL-15 induced T cell activation can be a new drug candidate for the treatment of RA. Therefore, we started our research program on new orally active anti-rheumatic drugs and found an inhibitor for IL-15 induced T cell activation as a new class of immunomodulator [8]. A novel piperidine-substituted compound **1** (Fig. (**1**)) showed an inhibitory effect on IL-15 dependent proliferation of CTLL-2 cells and ameliorated collagen-induced arthritis (CIA) in mice. Compound **1** was metabolized immediately to afford a 4-hydroxypiperidine derivative **2** that had a more potent inhibitory activity for IL-15 induced T cell activation compared to **1**, suggesting that some hydrophilic substituents could be installed at the 4th position of the aniline part without impairing *in vitro* activity. And, our results suggest that the mechanism of action of compounds **1** and **2** is clearly distinguishable from that of an active metabolite of leflunomide (**3**), A77 1726 (**4**) which inhibits dihydroorotate dehydrogenase, a key enzyme of pyrimidine biosynthesis [8]. Herein, we showed our approach to find a potent compound with both high *in vitro* activity and high bioavailability through further investigations.





Leflunomide (**3**)



A77 1726 (**4**)

Fig. (1). The structures of the phenylpyrazoleanilide derivatives **1**, **2**, leflunomide (**3**), and its active metabolite, A77 1726 (**4**)

CHEMISTRY

We selected the 4-hydroxypiperidine substituted derivative **2** (the active metabolite of the piperidine substituted compound **1**) as a new key compound for the development of potent immunomodulating agents, and we substituted other hydrophilic moieties at the 4th position of the piperidine moiety. Synthetic methods for the series of new anilides substituted by cyclic amines are described in Scheme **1**, **2**, **3**, and **4**. A general synthetic method for the aniline parts is shown in Scheme **1**. Piperidine (**6a**/**b**), 4-hydroxypiperidine (**6c**), 4-(2-hydroxyethyl)piperidine (**6d**), and ethyl isonipecotate (**6e**) are commercially available. To a solution of nitrobenzonitrile **5** in acetonitrile were added equimolar triethylamine and cyclic amine **6**. The mixed solution was kept stirring at boiling point for 2-3 hours. Some portion of water was added to the reaction solution to give adduct **7**, as a precipitate. Nitro intermediate **7** was reduced to give the aniline **8** according to the literature [9, 10].

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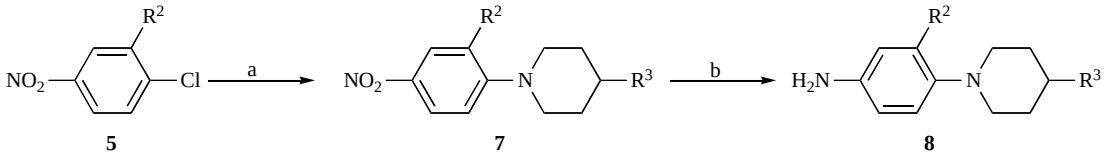
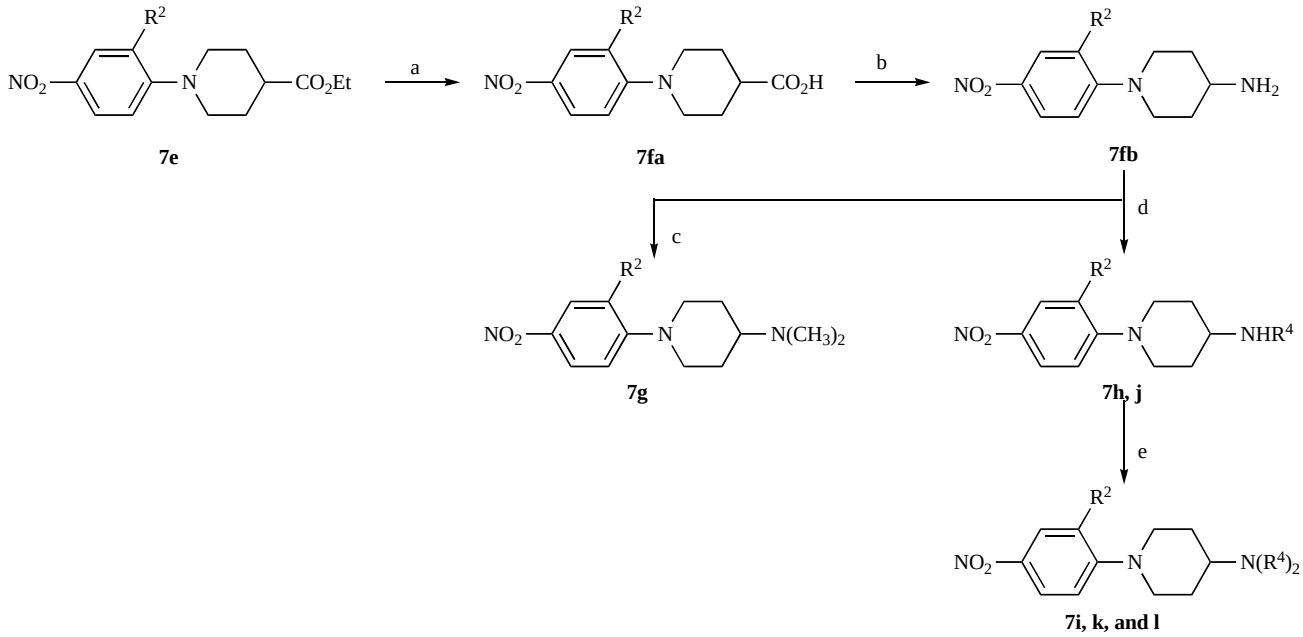


Scheme 1. Reagents and conditions: a) **6a-d**, Et_3N/CH_3CN, b) $FeCl_3$, activated charcoal, hydrazine/ MeOH, reflux, or Fe/ NH_4Cl/aq. EtOH.



Scheme 2. Reagents and conditions: a) NaOH/aq.EtOH, reflux; b) DPPA, Et_3N/tert-BuOH, reflux; then conc. HCl; c) HCHO/ HCO_2H, reflux; d) i: $(Boc)_2O$, Et_3N/ THF, r.t.; ii: NaH, then R^4-X/ DMF, 0 ˚C; iii: TFA, 0 ˚C; e) NaH, then R^4-X/ DMF, 0 ˚C.

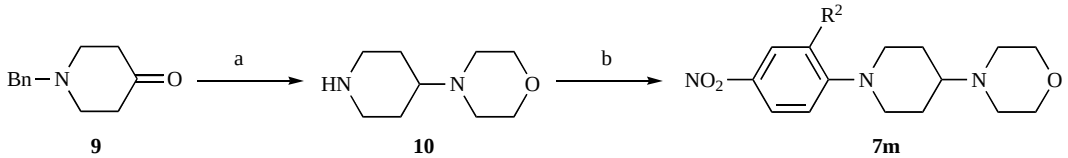
Synthetic methods for the primary and secondary amines substituted piperidine derivatives at the 4th position are shown in Scheme **2**. Isonipecotate adduct **7e** was hydrolyzed under basic conditions to give carboxylic acid **7fa**, which was treated with diphenylphospholylazide (DPPA) in *tert*-BuOH and was hydrolyzed under acidic conditions to give 4-aminopiperidine derivative **7fb**. **7fb** was treated with HCHO in HCO_2H to give 4-dimethylaminopiperidine derivative **7g**. And, **7fb** was protected by *tert*-butoxy carbonyl group and treated with NaH and alkyl halide (R^4-X), and then deprotected with trifluoroacetic acid (TFA) to give secondary amines (**7h** and **7j**), which afforded tertiary amines (**7i**, **7k**, and **7l**) by using NaH and alkyl halide under the same conditions as those described above.

The piperidine derivative **7m** having a morphline moiety was obtained through a reductive amination reaction of commercially available N-benzylpiperidone with morpholine in the presence of $NaBH(AcO)_3$ in toluene, depretection *via* hydrogenation reaction, and addition reaction to nitrobenzene **5** as shown in Scheme **3**.

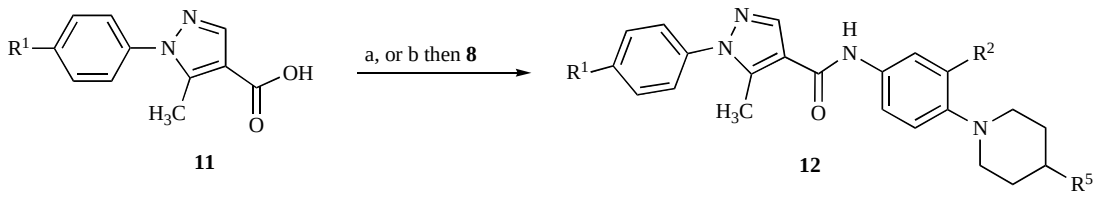
Finally, a series of anilides **12** was obtained by condensation of the acids **11** [8, 11] and the anilines **8** in N-methylpyrrolidone (NMP) *via* acid chloride by the treatment of $SOCl_2$. And, this condensation reaction proceeded using condensing agents, such as HOBt and WSC·HCl in DMF (Scheme **4**). Herein, we commonly used 1-phenyl-5-methylpyrazole-4-carboxylic acid derivatives **11**.

RESULTS AND DISCUSSION

In a primary *in vitro* assay, we evaluated the effects of compounds on proliferation of rat T cells stimulated with phorbol-12-myristate-13-acetate (PMA) and calcium ionophore (A23187) [12] (Table **1**). First of all, as to the substituents of the 4th position of the phenyl moiety in the carboxylic acid part, the conversion of F to Cl led to an increase



Scheme 3. Reagents and conditions: a) i: Morpholine, $NaBH(OAc)_3$/ toluene, r.t.; ii: Pd-C, H_2/EtOH, r.t.; b) **5**, Et_3N/ CH_3CN, reflux



Scheme 4. Reagents and conditions: a) i: $SOCl_2$ / toluene; ii: **8**/NMP; b) **8**, HOBt, WSC·HCl / DMF, r.t.

in *in vitro* activity (**1** to **12a**, and **2** to **12c**). Therefore, we selected the 1-(4-chlorophenyl)-5-methylpyrazole-4-carbonyl group as the standard unit, and then we evaluated the effects of substituents of the aniline part. Removal of the CN group at the 3rd position (R^2) of the aniline part decreased an *in vitro* activity (**12a** to **12b**). From these results, an electron deficient aniline unit should be necessary for an *in vitro* activity. By the extension of the distance between the piperidine ring and the terminal hydroxyl group, we obtained 4-(2-hydroxy)piperidine derivative **12d**, which had the most potent *in vitro* activity (9 nmol/L (IC_{50})). However, **12d** showed no clear effect on type II collagen-induced arthritis (CIA) in DBA/1J mice [13-15] (data not shown), presumably due to low solubility.

In order to improve solubility, we synthesized and evaluated other compounds having another type of hydrophilic substitutes, such as ethoxycarbonyl (**12e**), hydroxycarbonyl (**12f**), dimethylamino (**12g**) groups. Unfortunately, *in vitro* activities of all these compounds were diminished. From these results, we realized the importance of the alcohol-type oxygen atom at the terminal of the aniline part. Next, aminoalcohol compounds were evaluated to find that secondary aminoalcohol moities or aminoether ones were preferable and compounds with N-bis(alkyl)amino groups (**12i**, **12k**, and **12l**) were more potent than ones with N(H)alkylamino groups (**12h** and **12j**) in *in vitro* activity. Among them, bis(2-methoxyethyl)amine derivative **12l** had a potent *in vitro* activity (19 nmol/L (IC_{50})). Then, we reached an optimal cyclic amine having terminal ether-type-oxygen, that is, 4-(morpholin-4-yl)piperidine derivative, **12m** (Y-320) which had the most potent inhibitory activity (18 nmol/L (IC_{50})) on proliferation of rat T cells induced by PMA and Ca ionophore. Table **2** shows the pharmacokinetic property of Y-320 in rats.

Table **3** shows the comparison of the inhibitory effects of Y-320 with pyrazoleanilide **1**, **2**, leflunomide (**3**), and A77 1726 (**4**) on proliferation of CTLL-2 cells induced by recombinant mouse IL-15 [16-20]. As described, Y-320 had a potent inhibitory activity on IL-15-dependent proliferation of CTLL-2 cells and its activity was about 160-times more potent than that of A77 1726 (**4**).

Finally, we evaluated the effect of Y-320 on mouse CIA by orally administration at does of 0.3, 1 and 3 mg/kg for 35 days after primary immunization (Fig. (**2**)). More than 0.3 mg/kg of Y-320 significantly inhibited the arthritis in a dose-dependent manner and its inhibitory effect was more potent than that of leflunomide.

Table 1. The Inhibitory Activities for the Proliferation of Rat T Cells Induced by PMA and Ca Ionophore, and Solubility

Compds	R^1	R^2	R^5	IC_{50}[a] (μmol/L)	Solubility[b] (mg/mL)
1	F	CN	H	2.4	0.001
2	F	CN	OH	0.38	<0.001
A77 1726 (4)				0.54	NT
12a	Cl	CN	H	0.44	NT
12b	Cl	H	H	72	NT
12c	Cl	CN	OH	0.08	NT
12d	Cl	CN	CH_2CH_2OH	0.009	<0.001
12e	Cl	CN	CO_2Et	4.2	NT
12f	Cl	CN	CO_2H	30	NT
12g	Cl	CN	$N(CH_3)_2$	2.3	NT
12h	Cl	CN	$N(H)CH_2CH_2OH$	1.5	NT
12i	Cl	CN	$N(CH_2CH_2OH)_2$	0.43	NT
12j	Cl	CN	$N(H)CH_2CH_2OCH_3$	0.98	NT
12k	Cl	CN	$N(CH_3)CH_2CH_2OCH_3$	0.21	NT
12l	Cl	CN	$N(CH_2CH_2OCH_3)_2$	0.019	NT
Y-320 (12m)	Cl	CN	Morpholin-4-yl	0.018	0.01

[a]IC_{50} values represent an average of at least three independent experiments. [b]Solubility of compound in buffer solution at pH 1.2.

Table 2. Pharmacokinetic Parameters of Y-320 (12m) in SD-IGS Male Rats (n=4)

Dose (mg/kg)	Route	Tmax (h)	Cmax (ng/mL)	AUC_{1-24} (ng·h/mL)	T1/2 (h)	BA (%)
3	i.v.	-	-	1526 ± 267	2.0 ± 0.1	-
10	p.o.	3.0 ± 1.2	381 ± 136	2401 ± 880	2.3 ± 0.2	47.2

Table 3. IC_{50} Values of the Pyrazoleanilide 1, 2, and Y-320 (12m), Leflunomide (3), and A77 1726 (4)

Compd.	IC_{50} (nmol/L) (95% confidence interval)	
1	972	(939 – 994)
2	1340	(1090 – 2260)
Leflunomide (3)	15300	(13000 – 178000)
A77 1726 (4)	14700	(12600 – 17100)
Y-320 (12m)	90.0	(67.8 – 96.9)

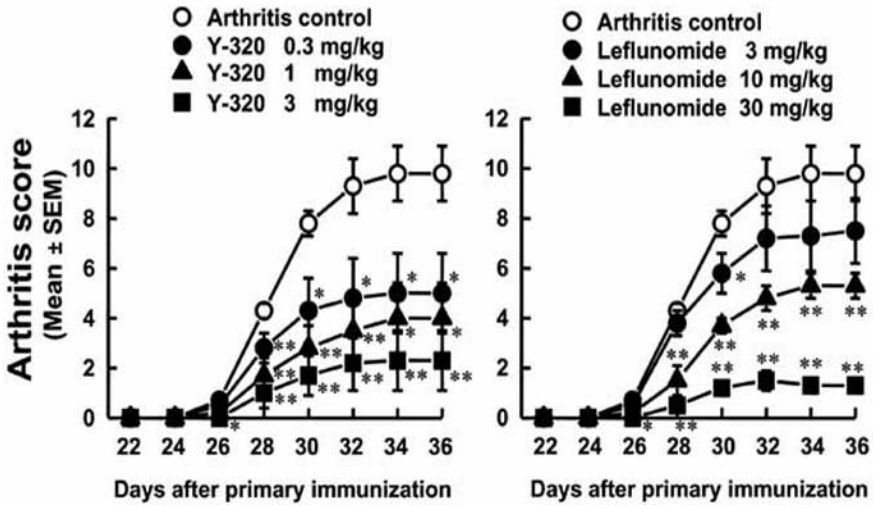


Fig. (2). Prophylactic effects of Y-320 (**12m**) and leflunomide (**1**) on CIA in DBA/1J mice.

CIA in DBA/1J mice was induced by immunization with 200 μg/mouse of bovine type II collagen and Freund's complete adjuvant on day 0 and 21. Y-320 and leflunomide were orally administered to mice from the day of primary immunization. Each column represents the mean ± SEM of 5 mice. Statistical differences were calculated by Dunnett's multiple comparison test (*: $p<0.05$, **: $p<0.01$ versus vehicle-treated control group).

CONCLUSION

Through the optimization of the active metabolite **2** of the piperidine derivative **1** as a basic skeleton, we found a new phenylpyrazoleanilide Y-320, which inhibits T cell activation induced by IL-15 and shows a therapeutic effect on type II collagen-induced arthritis in DBA/1J mice. Because IL-15 appears to play an important role in the pathogenesis of RA, it is highly probable that Y-320, a new class of immunomodulator, is a promising drug candidate for the treatment of RA.

REFERENCES AND NOTES

[1] Duke, O.; Panayi, G. S.; Janossy; G., Poulter, L. *Clin. Exp. Immunol.,* **1982**, *49*, 22.

[2] Thomas, R.; Mcllraith, M.; Davis, L. S.; Lipsky, P. E. *Arthritis Rheum*., **1992**, *35*, 1455.

[3] Iannone, F.; Corrigall, V. M.; Kingsley, G.; Panayi, G. *Eur. J. Immunol.,* **1994**, *24*, 2706.

[4] McInnes, I. B.; Al-Mughales, J.; Field, M.; Leung, B. P.; Huang, F. P.; Dixon, R.; Sturrock, R. D.; Wilkinson, P. C.; Liew, F. Y. *Nat. Med.,* **1996**, 2, 175.

[5] McInnes, I. B.; Leung, B. P.; Sturrock, R. D.; Field, M.; Liew. F. Y. *Nat. Med.,* **1997**, *2*, 189.

[6] Zoilkowska, M.; Koc, A.; Luszczykiewics, G.; Ksiesopolska-Pietrzak, K.; Kilmczak, E.; Chwalinska-Sadowska, H.; Maslinski, W. *J. Immunol.,* **2000**, *164*, 2832.

[7] Lubberts, E.; Koenders, M. I.; van den Berg, W. B. *Arthritis Res. Ther*., **2005**, *7*, 29.

[8] Ushio, H.; Ishibuchi, S.; Adach, K.; Oshita, K.; Chiba, K. *Lett. Drug Des. Discov*., **2008**, *5*, 111.

[9] $FeCl_3/H_2N\text{-}NH_2$ condition for benzonitrile derivatives, see: Hine, J.; Hahn, S.; Miles, D. E.; Ahn, K. *J. Org. Chem.,* **1985**, *50*, 5092.

[10] Fe (powder)/ NH_4Cl condition, see: Tsuji, K.; Nakamura, K.; Konishi, N.; Okuma, H.; Matsuo, M. *Chem. Pharm. Bull.,* **1992**, *40*, 2399.

[11] Wang, A. X.; Xie, Q.; Lane, B.; Mollison, K. W.; Hsieh, G. C.; Marsh, K.; Cheets, M. P.; Luly, J. R.; Coghlam, M. J. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 2787.

[12] Splenic T cells (2.5×105 cells/well) from F344 rats were stimulated with 10 ng/mL PMA and 100 ng/mL A23187 in the presence or absence of test compounds using RPMI 1640 medium containing 10% fetal calf serum (FCS) and 5×10^{-5} M of 2-mercaptoethanol (2-ME) in 96-well microtest plates. After culturing for 44 h, the cells were pulsed with 18.5 kBq/well [methyl-^{3}H]thymidine ([^{3}H]TdR, Amersham Pharmacia Biotec) for an additional 4 h. Radioactivity incorporated into the cells was determined by scintillation counter (1450 MicroBetaSystem).

[13] Typical procedure: see: footnote in Fig. (**2**).

[14] Paska, W.; McDonald, K. J.; and Croft. M. *Agent Action*, **1986**, *18*, 413.

[15] Nemoto, K.; Mae, T.; Abe, F.; Takeuchi, T. *Immunomodulating Drugs*, **1993**, *685*, 148.

[16] Kamada, H. and Nakagami, K. *Jpn. J. Pharmacol.*, **1996**, *70*, 169.

[17] Elder, R. T.; Xu, X.; Williams, J. W.; Gong, H.; Finnegan, A.; and Chong, A. S.-F. *J. Immunol.*, **1997**, *159*, 22.

[18] Ruchatz, H.; Leung, B. P.; Wei, X.; McInnes, I. B.; Liew, F. Y. *J. Immunol.*, **1998**, *160*, 5654.

[19] Gillis, S.; Ferm, M. M.; Ou, W.; Smith, K. A. *J. Immunol.*, **1978**, *120*, 2027.

[20] CTLL-2 cells (104 cells/well) stimulated with 100 ng/ml recombinant mouse IL-15 were cultured at 37 ˚C for 44 h in the presence or absence of tested compounds (0.0001-100 μM) using RPMI 1640 medium containing 10% FCS and 5×10^{-5} M of 2-ME in 96-well microtest plates. After the culturing, the cells were pulsed with 18.5 kBq/well [^{3}H]TdR for an additional 4 h and the radioactivity incorporated into the cells was determined as described above.