Drugs of the Future 2001, 26(1): 32-38

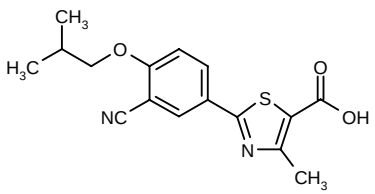
CCC: 0377-8282/2001

TMX-67

Treatment of Gout and Hyperuricemia
Xanthine Oxidase Inhibitor

TEI-6720

2-(3-Cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid



$C_{16}H_{16}N_2O_3S$ Mol wt: 316.3794

CAS: 144060-53-7

EN: 186290

Synthesis

TMX-67 can be obtained by several related ways:

1) Reaction of 4-hydroxy-3-nitrobenzaldehyde (I) with hydroxylamine and sodium formate in refluxing formic acid gives 4-hydroxy-3-nitrobenzonitrile (II), which is treated with thioacetamide in hot DMF to yield the corresponding thiobenzamide (III). The cyclization of (III) with 2-chloroacetoacetic acid ethyl ester (IV) in refluxing ethanol affords 2-(4-hydroxy-3-nitrophenyl)-4-methylthiazole-5-carboxylic acid ethyl ester (V), which is alkylated at the phenolic group by means of isobutyl bromide (VI) and K_2CO_3 in hot DMF, providing the isobutyl ether (VII). The reduction of the NO_2 group of (VII) with H_2 over Pd/C in ethanol/ethyl acetate gives the expected amino derivative (VIII), which is converted into 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid ethyl ester (IX) by diazotation with $NaNO_2$/HCl and treatment with CuCN and KCN. Finally, this compound is hydrolyzed with NaOH in hot THF/water (1). Scheme 1.

2) The reaction of 4-nitrobenzonitrile (X) with KCN in hot DMSO, followed by treatment with isobutyl bromide (VI) and K_2CO_3, gives 4-isobutoxybenzene-1,3-dicarbonitrile (XI), which is treated with thioacetamide in hot DMF to yield 3-cyano-4-isobutoxythiobenzamide (XII). Cyclization of (XII) with 2-chloroacetoacetic acid ethyl ester (IV) in refluxing ethanol affords 2-(3-cyano-4-isoutoxyphenyl)-4-methylthiazole-5-carboxylic acid ethyl ester (IX), which is hydrolyzed with NaOH as before (2, 3). Scheme 2.

3) Cyclization of 4-hydroxythiobenzamide (XIII) with 2-bromoacetoacetic acid ethyl ester (XIV) in refluxing ethanol provides 2-(4-hydroxyphenyl)-4-methylthiazole-5-carboxylic acid ethyl ester (XV) (4, 5), which is formylated by reaction with hexamethylenetetramine (HMTA) and polyphosphoric acid (PPA) in hot AcOH/water to afford 2-(3-formyl-4-hydroxyphenyl)-4-methylthiazole-5-carboxylic acid ethyl ester (XVI). Alkylation af (XVI) with isobutyl bromide (VI), K_2CO_3 and KI in DMF gives 2-(3-formyl-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid ethyl ester (XVII), which is treated with formic acid, sodium formate and hydroxylamine hydrochloride to give the already reported 2-(3-cyano-4-isobutoxyphenyl)4-methyl-thiazole-5-carboxylic acid ethyl ester (IX). Finally, this compound is hydrolyzed with NaOH in THF/EtOH (6). Scheme 3.

4) Alternatively, 2-(3-formyl-4-hydroxyphenyl)-4-methylthiazole-5-carboxylic acid ethyl ester (XVI) can also be treated first with formic acid, sodium formate and hydroxylamine hydrochloride to provide 2-(3-cyano-4-hydroxyphenyl)-4-methylthiazole-5-carboxylic acid ethyl ester (XVIII), which is then alkylated with isobutyl bromide (VI) as before to give the already reported 2-(3-cyano-4-isobutoxyphenyl)-4-methylthiazole-5-carboxylic acid ethyl ester (IX) (6). Scheme 3.

Description

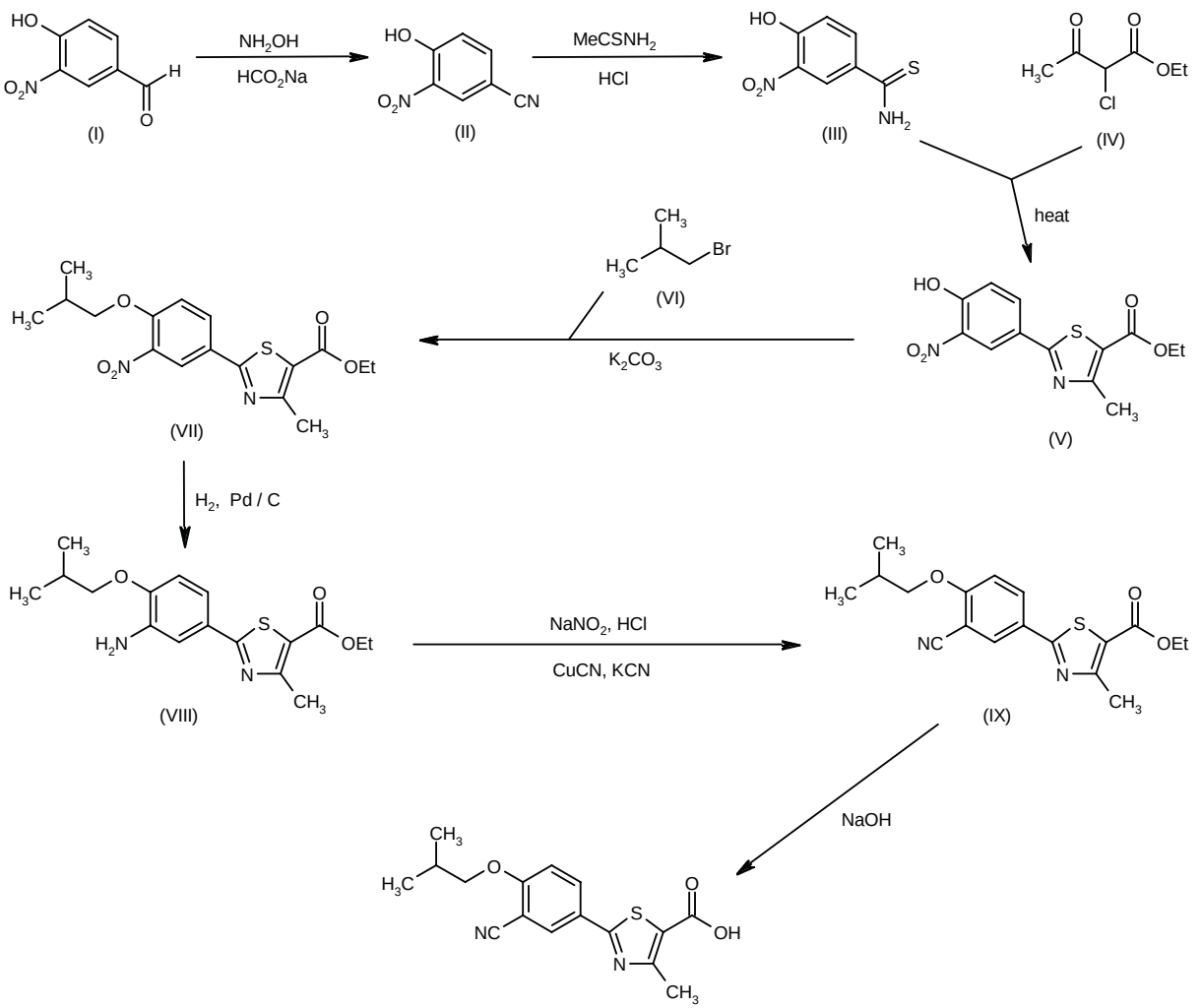
Colorless crystals, m.p. 201-2 °C (2).

Introduction

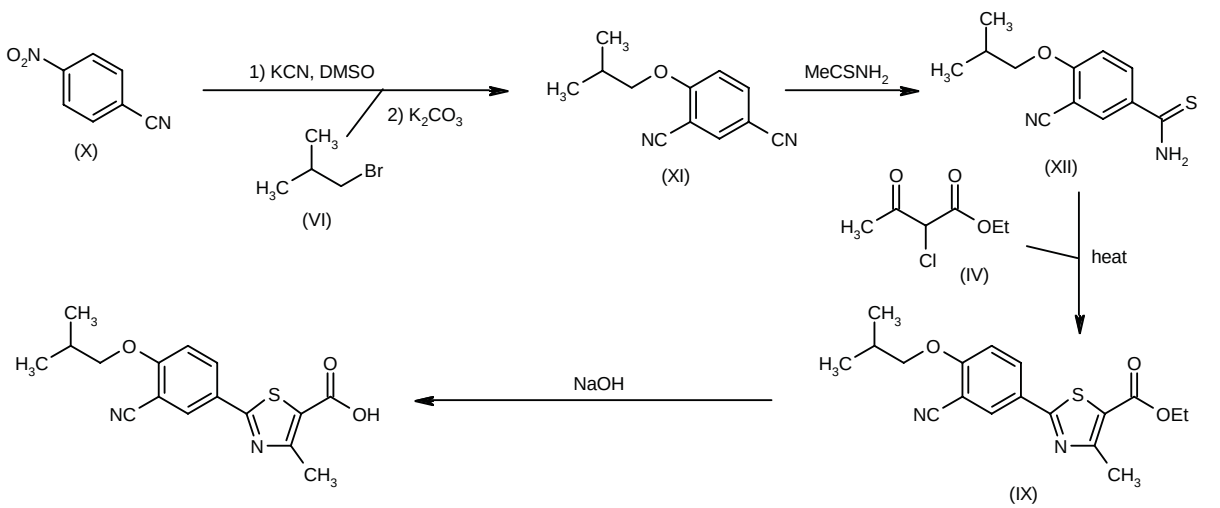
Hyperuricemia due to an increase in uric acid production and/or a decrease in renal uric acid secretion is considered the most significant risk factor for the development of gout (7). Treatment to prevent the onset of gout involves use of agents to reduce serum uric acid levels. Types of agents include the uricosurics (Table I) which block uric acid absorption at the luminal membrane of renal tubules, and xanthine oxidase/xanthine

L.A. Sorbera, L. Revel, X. Rabasseda, J. Castañer. Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of TMX-67



Scheme 2: Synthesis of TMX-67



Scheme 3: Synthesis of TMX-67

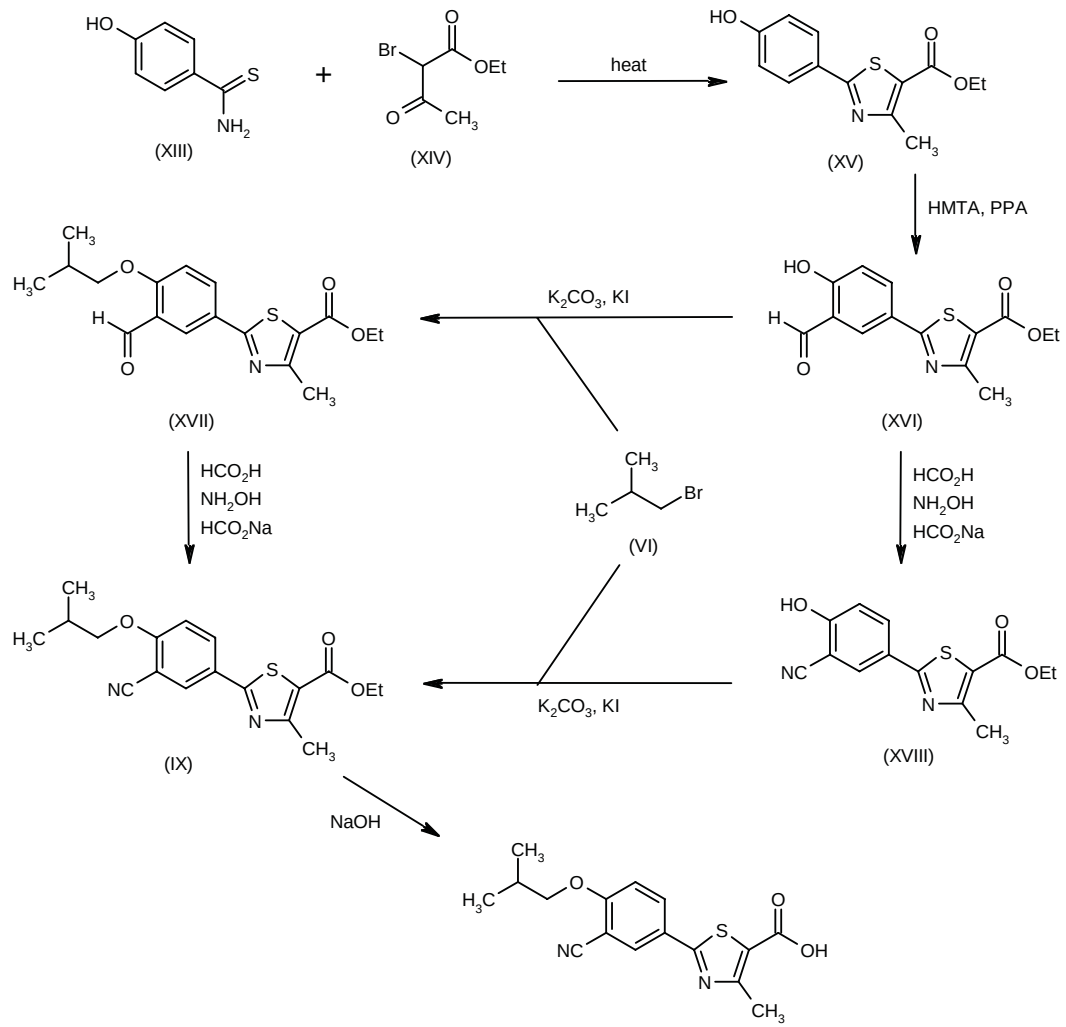
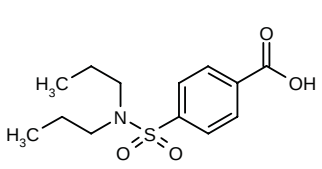
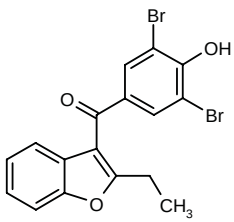


Table I: Compounds available for the treatment of gout (Prous Science Ensemble database).

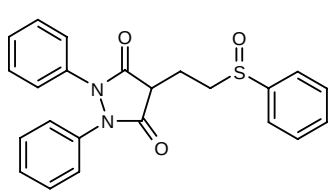
Drug Name	Company	Status
Uricosurics		
1. Probenecid	Merck & Co.	Launched 1951
2. Benzbromarone	Sanofi-Synthélabo	Launched 1976
3. Sulfinpyrazone	Novartis	Launched 1959
Xanthine Oxidase Inhibitor		
4. Allopurinol	GlaxoSmithKline	Launched 1967



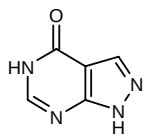
(1)



(2)



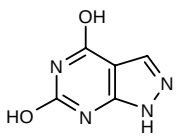
(3)



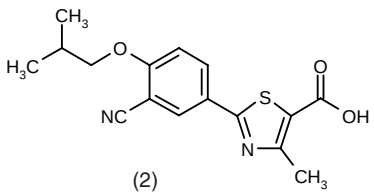
(4)

Table II: Compounds under investigation for the treatment of gout (Prous Science Ensemble database).

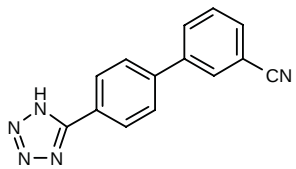
Drug Name	Company	Status
Xanthine Oxidase Inhibitors		
1. Oxipurinol	Ilex Oncology	Phase II
2. TMX-67	Teijin/TAP Pharm.	Phase II/I
3. KT-651	Kotobuki	Preclinical
4. Y-700	Welfide	Preclinical
Recombinant Urate Oxidase Inhibitor		
5. Rasburicase	Sanofi-Synthélabo	Preregistered



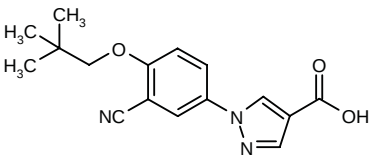
(1)



(2)



(3)



(4)

Recombinant urate oxidase

(5)

Table III: Comparative inhibitory constants (IC_{50}) against xanthine oxidase for various drugs (Prous Science MFline database).

Drug	IC_{50}	Ref.
Allopurinol	1.7-4 μM	10, 23
Hydroxyakalone	4.6 μM	23
KT-651	20 nM	24
Piceatannol	>30 μM	25
Resveratrol	>30 μM	25
Rhapontigenin	34 μM	25
TMX-67	1.4 nM	10, 26
VB-5122	22 nM	27
Y-700	4.3-6.5 nM	28

dehydrogenase inhibitors. Unfortunately, uricosuric agents cannot be used in individuals with renal dysfunction and administration to those patients with normal renal function requires alkalization of urine. As for xanthine oxidase/ xanthine dehydrogenase inhibitors, only 1 agent, allopurinol, is commercially available. Allopurinol, however, is administered only to patients in whom uricosuric agents failed to reduce serum uric acid below 7 mg/dl, to patients intolerant of uricosurics or to patients with gout due to increased uric acid production and with renal dysfunction (8). Moreover, allopurinol is associated with significant adverse effects such as hepatitis, nephropathy and allergic reactions (9).

Since inhibition of xanthine oxidase is the mechanism of choice for prevention of the onset of gout, researchers have focused on development of agents with a favorable safety profile as compared to allopurinol (Table II). In the search for new anti-gout agents, the newly synthesized TMX-67 emerged showing excellent activity and has been chosen for further development.

Pharmacological Actions

TMX-67 inhibited bovine milk xanthine oxidase and mouse and rat liver xanthine oxidase/xanthine dehydrogenase with IC_{50} values of 1.4, 1.8 and 2.0 nM, respectively; IC_{50} values obtained for allopurinol for the same substrates were 1700, 380 and 1100 nM, respectively (Table III). TMX-67-induced inhibition of bovine milk xanthine oxidase was of a mixed type (K_i = 0.7 nM) as compared to inhibition with allopurinol which was competitive (K_i = 280 nM) (10).

Further *in vitro* studies using a lung cancer cell line (A549) demonstrated that TMX-67 (16 μM for 3 h) completely inhibited xanthine oxidase activity without affecting the activities of adenosine deaminase, purine nucleoside phosphorylase, adenine phosphoribosyltransferase, hypoxanthine guanine phosphoribosyltransferase, pyrimidine nucleoside phosphorylase or guanase. Exposure of cells in inosine- or uridine-containing media to TMX-67 prevented the reductions in extracellular inosine and uridine, respectively. Results also showed that the agent noncompetitively inhibited Na-dependent transport of uridine with a K_i value of 4.1 μM (11).

The hypouricemic effect of TMX-67 was demonstrated *in vivo* in rodent and chimpanzee models. Orally administered TMX-67 was more potent than allopurinol

(ED_{50} = 0.7 *vs.* 2.7 mg/kg 2 h postdosing in mice) in lowering serum urate levels in normal rats and mice and the duration of TMX-67 action was longer than that of allopurinol. Repeated TMX-67 dosing (1-100 mg/kg p.o. once daily for 28 days) in normal rats was found to be approximately 10- to 30-fold more potent than allopurinol (3-200 mg/kg p.o. once daily) in increasing plasma and urinary xanthine levels; no differences in xanthine excretion rates or renal calculus formation were observed between the two agents. TMX-67 was also effective in rats with potassium oxonate (250 mg/kg s.c. 1 h before TMX-67)-induced hyperuricemia. Both TMX-67 and allopurinol were hypouricemic 2 h postdosing with ED_{50} values of 1.5 and 5 mg/kg p.o., respectively; both agents decreased the molarity of serum uric acid and allantoin with ED_{50} values of 2.1 and 6.9 mg/kg p.o., respectively (10, 12).

The hypouricemic efficacy of TMX-67 (5 g/kg/day p.o. for 3 days) was also shown in an *in vivo* study conducted in chimpanzees where the agent was found to be more potent than allopurinol (10 mg/kg/day p.o. once daily for 3 days). TMX-67 decreased serum urate levels by 55.9, 69.6 and 73.6% at 24, 48 and 72 h after the first dose as compared to decreases of 28.1, 41.6 and 45.1%, respectively, seen after allopurinol administration. Overall decreases in total uric acid content were 96.5% for TMX-67 as compared to 78.6% for allopurinol (13).

Several safety and toxicity studies have been performed comparing TMX-67 and allopurinol. A study using a mouse contact hypersensitivity model showed that while allopurinol (30-100 mg/kg/day p.o.) dose- and time-dependently increased mortality and significantly increased ear swelling in dinitrofluorobenzene (DNFB; topically)-sensitized mice, TMX-67 (3, 10, 30 or 100 mg/kg/day p.o.) had little effect. Moreover, allopurinol (30 mg/kg/day) significantly reduced spleen weight, body weight (–4.2 ± 0.6 *vs.* –2.0 ± 0.3 g) and white blood cell counts (3.9 ± 0.5 million/ml *vs.* 5.2 ± 0.3 million/ml) as compared to control mice. In contrast, TMX-67 had no significant effect on body weight or white blood cell counts; a dose of 30 mg/kg did significantly reduce spleen weight although to a lesser extent than allopurinol and further reductions in weight were not observed with the 100 mg/kg dose (14).

Other studies also using DNFB-sensitized mice reported superior results for TMX-67 over allopurinol. While allopurinol (30 or 100 mg/kg/day) induced a variety of toxic effects including increases in plasma GPT, GOT, creatinine and BUN, increases in plasma and urinary orotidine (indicating abnormal pyrimidine metabolism), decreases in the percent of polychromatic erythrocytes of bone marrow cells, increased calculus formation in collecting tubules and papillary ducts in sensitized-mice and induction of renal impairment in nonsensitized and sensitized mice, TMX-67 (30 or 100 mg/kg/day) had no toxic effects (15, 16).

The safety of TMX-67 was shown from results obtained from a female rhesus monkey administered the agent orally (100 mg/kg) for 4 days. No abnormal changes were detected in blood or urine parameters, body weight. or temperature (13).

Pharmacokinetics

A study examined the absorption and excretion of TMX-67 in male rats following oral (1, 3 or 10 mg/kg) and i.v. (0.5 mg/kg) administration of the [^{14}C]-labeled compound. Radioactivity was maximum (1.97 μg eq·/ml) at 15 min after oral dosing with 1 mg/kg, after which levels decreased triphasically, with $t_{1/2\alpha}$, $t_{1/2\beta}$ and $t_{1/2\gamma}$ values of 8.6 min, 3.1 h and 57.1 h, respectively. Absolute bioavailability with this dose was 71%. The AUC and C_{max} values dose-dependently increased. Plasma unchanged drug levels were 80% of the radioactivity 8 h following both p.o. and i.v. dosing. Rapid elimination of the agent with no accumulation was suggested, since the plasma concentration of the agent did not vary following single or multiple oral dosing (14 doses). At 168 h after oral and i.v. dosing, 37 and 48%, respectively, of the radioactivity was recovered in urine and 59 and 47%, respectively, was recovered in feces. In bile duct-cannulated rats, 52% of the radioactivity was excreted in bile following oral administration. The terminal $t_{1/2}$ ratios of partially nephrectomized to sham rats after i.v. administration of [^{14}C]-TMX-67 or [^{14}C]-allopurinol (0.5 mg/kg) were 1.6 and 4.9, respectively, due to the difference in urinary excretion of the two agents, thus indicating that TMX-67 is safer for patients with renal impairment (17).

A study conducted in rats examining the metabolism of [^{14}C]-TMX-67 following oral administration reported that the percentage of unchanged drug in bile was 15% with several polar metabolites (including a glucuronide or sulfate of TMX-67) also detected, and 10% in urine with additional glucuronidase-resistant metabolites. Further metabolism results *in vitro* revealed slow P-450-mediated oxidation or glucuronidation of the agent in rat and human microsomes (18).

The tissue distribution and *in vitro* protein binding in rat serum and human plasma of the agent were described in rats following oral administration of [^{14}C]-TMX-67 (1 mg/kg). While radioactivity in the gastrointestinal tract, liver and kidney was higher than in plasma at 1 h postdosing, at 168 h postdosing no radioactivity was detected in tissue. The maximum level of radioactivity reached in liver was 1.06 μg eq·/g at 1 h postdosing which decreased to 0.11 μg eq·/g at 24 h. Results from *in vitro* studies reported a protein binding rate for the agent of 99% in both rat serum and human plasma, with albumin being the major protein (18).

Interestingly, an HPLC method was developed to determine human plasma purine nucleoside phosphorylase activity which uses TMX-67. This method was concluded to be more effective than existing spectrophotometric methods or HPLC methods incorporating xanthine oxidase (19).

Clinical Studies

An ongoing double-blind, placebo-controlled, escalating, multiple-dose study is examining the safety and

efficacy of TMX-67 (10, 20, 30, 40 and 50 mg on day 1 and days 3-14) in 12 healthy volunteers. The agent was well tolerated with few adverse events, of which the majority were mild. Mean serum uric acid levels at 24 h decreased by approximately 27, 34, 37, 40 and 47% for the respective doses. Linear pharmacokinetics were obtained (20).

TMX-67 is currently undergoing phase II trials in Japan by Teijin and phase I trials in the U.S. by TAP Pharmaceuticals for the treatment of hyperuricemia and gout (21, 22).

Manufacturer

Teijin Ltd. (JP) licensed to TAP Pharmaceuticals Inc. (US) for the U.S. and Canada.

References

1. Kondo, S., Fukushima, H., Hasegawa, M., Tsuchimoto, M., Nagata, I., Osada, Y., Komoriya, K., Yamaguchi, H. (Teijin Ltd.). *2-Arylthiazole deriv. and pharmaceutical compsn. containing the same.* EP 0513379, JP 1993500083, US 5614520, WO 9209279.

2. Hasegawa, M. *A facile one-pot synthesis of 4-alkoxy-1,3-benzenedicarbonitrile.* Heterocycles 1998, 47: 857-64.

3. Hasegawa, M., Komoriya, K. (Teijin Ltd.). *Cyano cpds. and their preparation method.* JP 1994345724.

4. Miller, B. (American Cyanamid Co.). *Thiazolylphenyl phosphates.* US 3518279.

5. Watanabe, K., Tanaka, T., Kondo, S., Fujii, T. (Teijin Ltd.). *Preparation method of 2-(4-alkoxy-3-cyanophenyl)thiazol derivs. and novel intermediates for their synthesis.* JP 1994329647.

6. Watanabe, K., Yarino, T., Hiramatsu, T. (Teijin Ltd.). *Preparation method of 2-(4-alkoxy-3-cyanophenyl)thiazole derivs.* JP 1998045733.

7. Campion, E.W., Glynn, R.J., DeLabry, L.O. *Asymptomatic hyperuricemia: Risks and consequences in the Normative Aging Study.* Am J Med 1987, 82: 421-6.

8. Kelley, W.N., Schumacher, H.R. Jr. *Crystal-associated synovitis.* In: Textbook of Rheumatology, Vol. 2, W.N. Kelley, Jr., E.D. Harris, S. Ruddy, C.B. Sledge (Eds.). W.B. Saunders Co.: Philadelphia 1993, 1291.

9. Hande, K.R., Noone, R.M., Stone, W.J. *Severe allopurinol toxicity: Description and guidelines for prevention in patients with renal insufficiency.* Am J Med 1984, 76: 47-56.

10. Osada, Y., Tsuchimoto, M., Fukushima, H., Takahashi, K., Kondo, S., Hasegawa, M., Komoriya, K. *Hypouricemic effect of the novel xanthine oxidase inhibitor, TEI-6720, in rodents.* Eur J Pharmacol 1993, 241: 183-8.

11. Yamamoto, T., Moriwaki, Y., Fujimura, Y., Takahashi, S., Tsutsumi, Z., Tsutsui, T., Higashino, K., Hada, T. *Effect of TEI-6720, a xanthine oxidase inhibitor, on the nucleoside transport in the lung cancer cell line A549.* Pharmacology 2000, 60: 34-40.

12. Horiuchi, H., Ota, M., Kobayashi, M., Kaneko, H., Kasahara, Y., Nishimura, S., Kondo, S., Komoriya, K. *A comparative study on the hypouricemic activity and potency in renal xanthine calculus formation of two xanthine oxidase/xanthine dehydrogenase inhibitors: TEI-6720 and allopurinol in rats.* Res Commun Mol Pathol Pharmacol 1999, 104: 307-19.

13. Komoriya, K., Osada, Y., Hasegawa, M., Horiuchi, H., Kondo, S., Couch, R.C., Griffin, T.B. *Hypouricemic effect of allopurinol and the novel xanthine oxidase inhibitor TEI-6720 in chimpanzees.* Eur J Pharmacol 1993, 250: 455-60.

14. Horiuchi, H., Ota, M., Kitahara, S., Ohta, T., Kiyoki, M., Komoriya, K. *Allopurinol increases ear swelling and mortality in a dinitrofluorobenzene-induced contact hypersensitivity mouse model.* Biol Pharm Bull 1999, 22: 810-5.

15. Horiuchi, H., Ota, M., Kaneko, H., Kasahara, Y., Ohta, T., Komoriya, K. *Nephrotoxic effects of allopurinol in dinitrofluorobenzene-sensitized mice: Comparative studies on TEI-6720.* Res Commun Mol Pathol Pharmacol 1999, 104: 293-305.

16. Horiuchi, H., Ota, M., Nishimura, S., Kaneko, H., Kasahara, Y., Ohta, T., Komoriya, K. *Allopurinol induces renal toxicity by impairing pyrimidine metabolism in mice.* Life Sci 2000, 66: 2051-70.

17. Nishimura, S., Mochizuki, T., Hoshide, S., Nagao, T., Ishii, S., Kondo, S., Kiyoki, M. *Metabolic fate of [^{14}C]-TEI-6720, a novel xanthine oxidase inhibitor: Absorption and excretion after intravenous and oral administration in rats.* 4th Int ISSX Meet (Aug 27-31, Seattle) 1995, Abst 302.

18. Kondo, S., Nishimura, S., Mochizuki, T., Taniguchi, K., Hoshide, S., Nagao, T., Ishii, S., Kiyoki, M. *Metabolic fate of [^{14}C]-TEI-6720, a novel xanthine oxidase inhibitor: Tissue distribution after oral administration in rats, protein binding and metabolism in vivo and in vitro.* 4th Int ISSX Meet (Aug 27-31, Seattle) 1995, Abst 56.

19. Yamamoto, T., Moriwaki, Y., Takahashi, S., Nasako, Y., Yamakita, J., Hiroishi, K., Higashino, K. *Determination of plasma purine nucleoside phosphorylase activity by high-performance liquid chromatography.* Anal Biochem 1995, 227: 135-9.

20. Khosravan, R., Bopp, B.A., Cox, M.A., Dean, R.R., Wilson, R.R., Weston, I.E. *Dose-related decreases in uric acid observed in a multiple-dose safety, pharmacokinetic, and pharmacodynamic study of TMX-67, a novel xanthine oxidase/dehydrogenase inhibitor, in healthy subjects.* 64th Annu Meet Am Coll Rheumatol (Oct 29-Nov 2, Philadelphia) 2000, Abst 2009.

21. Teijin and TAP *Holdings sign license agreement for gout therapeutic agent TMX-67.* Teijin Ltd. Press Release October 6, 1999.

22. *TMX-67 development status.* Teijin Ltd. Company Communication December 13, 2000.

23. Izumida, H., Adachi, K., Mihara, A., Yasuzawa, T., Sano, H. *Hydroxyakalone, a novel xanthine oxidase inhibitor produced by a marine bacterium, Agrobacterium aurantiacum.* J Antibiot 1997, 50: 916-8.

24. Koide, N., Katada, N., Kosakai, K., Suzaka, H., Tomiyama, A. *KT651, a new type of hypouricemic agent, has a inhibition on the uric acid biosynthesis and acceleration on uric acid excretion.* Jpn J Pharmacol 2000, 82(Suppl. 1): Abst P-525.

25. Matsuda, H., Morikawa, T., Toguchida, I., Kageura, T., Oda, M., Yoshikawa, M. *NO production inhibitory and antioxidant constituents from rhubarb - Structural requirements of stilbenes for the activity of plausible mechanism of action.* 20th Symp Med Chem (Dec 6-8, Tokyo) 2000, Abst 1P-24.

26. Osada, Y., Hukushima, H., Hasegawa, M., Kondo, S., Komoriya, K. *Hypouricemic effect of a novel xanthine oxidase (XOD) inhibitor TEI-6720 in rodents.* Jpn J Pharmacol 1993, 61(Suppl. I): Abst P-593.

27. van der Goot, H., Voss, H.-P., Bast, A., Timmerman, H. *New antioxidants with strong free radical scavenging and xanthine oxidase inhibiting activity.* 15th EFMC Int Symp Med Chem (Sept 6-10, Edinburgh) 1998, Abst P.243.

28. Fukunari, A., Inoue, H., Aoki, K., Horikama, T., Ishibuchi, S., Morimoto, H., Ikebe, T., Oe, T., Naka, Y., Okumoto, T. *Hypouricemic activity of Y-700, a new class of xanthine oxidase inhibitor.* Naunyn-Schmied Arch Pharmacol 1998, 358(1, Suppl. 2): Abst P 52.32.

Additional References

Hasegawa, M., Kondo, S., Fukushima, H., Tsuchimoto, M., Osada, Y., Komoriya, K., Yamaguchi, H. *2-Phenyl-5-thiazolecarboxylic acid derivatives: A new class of potent xanthine oxidase inhibitors.* 12th Int Symp Med Chem (Sept 13-17, Basel) 1992, Abst P-168.B.

Horiuchi, H., Ota, M., Kitahara, S., Ohta, T., Kiyoki, M., Komoriya, K. *Toxic effects of allopurinol on a contact hypersensitivity mouse model.* Jpn J Pharmacol 1999, 79(Suppl. I): Abst P-693.

Horiuchi, H., Ota, M., Kobayashi, M., Kaneko, H., Kasahara, Y., Nishimura, S., Kondo, S., Komoriya, K. *Hypouricemic activity of a novel xanthine oxidase/xanthine dehydrogenase (XOD/XDH) inhibitor, TEI-6720 in rats.* Jpn J Pharmacol 2000, 82(Suppl. 1): Abst P-606.

Moriwaki, Y., Yamamoto, T., Yamakita, J., Takahashi, S., Higashino, K. *Enzymatic histochemical localization of aldehyde oxidase in rat liver by the tetrazolium method.* Acta Histochem Cytochem 1997, 30: 113-5.