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International Journal of Pharmaceutics 158 (1997) 203–210

international journal of pharmaceutics

Multi-objective simultaneous optimization based on artificial neural network in a ketoprofen hydrogel formula containing *O*-ethylmenthol as a percutaneous absorption enhancer

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Received 24 April 1997; received in revised form 18 July 1997; accepted 2 August 1997

Abstract

The aim of this study was to apply a novel simultaneous optimization technique incorporating an artificial neural network (ANN) to a design of a ketoprofen hydrogel containing *O*-ethylmenthol (MET). For model formulae, 12 kinds of ketoprofen hydrogels were prepared. The amount of ethanol and MET were selected as causal factors. A percutaneous absorption study in vivo in rats was performed and irritation evoked on rat skin was microscopically judged after the end of the experiments. The rate of penetration (R_p), lag time (t_L) and total irritation score (TIS) were selected as response variables. A set of causal factors and response variables was used as tutorial data for ANN and fed into a computer. Nonlinear relationships between the causal factors and the release parameters were represented well with the response surface predicted by ANN. The optimization of the ketoprofen hydrogel was performed according to the generalized distance function method. The observed results of R_p and TIS, which had a lot of influence on the effectiveness and safety, coincided well with the predictions. It was suggested that the multi-objective simultaneous optimization technique incorporating ANN was quite useful for optimizing pharmaceutical formulae when pharmaceutical responses were nonlinearly related to the formulae and process variables. © 1997 Elsevier Science B.V.

Keywords: Multi-objective simultaneous optimization; Artificial neural networks; Transdermal absorption; *O*-ethylmenthol; Hydrogel

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PII S0378-5173(97)00260-3

1. Introduction

Many studies have discussed percutaneous absorption enhancers and the mechanisms of their enhancing activity (Barry, 1987; Okamoto et al., 1988; Kadir and Barry, 1991). Recently, we synthesized *O*-alkylmenthol and *O*-acylmenthol derivatives and investigated their enhancing activities for the percutaneous absorption of ketoprofen from the hydrogel in rats (Negishi et al., 1995; Nakamura et al., 1996). It was observed that *O*-ethylmenthol (MET) was the most promising compound with a great promoting action and relatively low skin irritancy. For an efficacious transdermal gel formula, we want to use sufficient amounts of enhancer and solvent to obtain a favorable drug absorption. At the same time, the vehicle components affecting the drug absorption should be kept as low as possible to avoid skin irritation. From the above reviewed data, it is not obvious how to choose the optimal formula. A response surface method (RSM) has often been applied to solving an optimization problem in pharmaceutical formulations (Shirakura et al., 1991; Takayama and Nagai, 1991; Levison et al., 1994; Ohara et al., 1996). However, prediction of pharmaceutical responses based on the second-order polynomial equation (PNE), which is commonly used in RSM, is often limited to a very low level, resulting in poor estimates of optimal formulae. In order to overcome the shortcomings in RSM, we recently developed a novel optimization technique in which an artificial neural network (ANN) was incorporated. A multi-objective simultaneous optimization of sustained-release tablet formulae containing Trapidil was successfully achieved using this technique (Takahara et al., 1997). ANN is a learning system based on a computational technique which attempts to simulate the neurological processing ability of the human brain (Achanta et al., 1995). Theoretical details of a hierarchical ANN has been given elsewhere. Briefly, the ANN has one input layer, one or more hidden layers and one output layer. Each layer has some units. The units in neighboring layers are fully interconnected with links. The strengths of connections between two units are called 'weight'. ANN leans to an approximate non-linear relationship by a procedure called 'training', varying weight values. It has successfully been applied to solving various problems in pharmaceutical research field such as product development (Hussain et al., 1991; Takahara et al., 1997), estimating diffusion coefficients (Jha et al., 1995), predicting the mechanism of drug action (Weinstein et al., 1992) and predicting pharmacokinetic parameters (Hussain et al., 1993; Brier et al., 1995; Gobburu and Shelver, 1995; Smith and Brier, 1996).

Fig. 1 shows a typical flow of the optimization technique, where ANN is applied to a nonlinear prediction of response variables by a combination of causal factors. The multi-objective simultaneous optimization, the last process in Fig. 1, was performed according to the generalized distance function method (Khuri and Conlon, 1981; Takayama and Nagai, 1991):

$$S(\boldsymbol{X}) = (\sum[\{FD_i(\boldsymbol{X}) - FO_i(\boldsymbol{X})\}/\mathrm{SD}_i]^2)^{1/2} \qquad (1)$$

where $S(\boldsymbol{X})$ is the distance function generalized by the standard deviation, $\mathrm{SD_i}$, of the observed values for each response variable, $FD_\mathrm{i}(\boldsymbol{X})$ is the optimum values of each response optimized individually over the experimental region and $FO_\mathrm{i}(\boldsymbol{X})$ is the simultaneous optimum value. The simultaneous optimum can be estimated by minimizing $S(\boldsymbol{X})$ under the restriction of the experimental

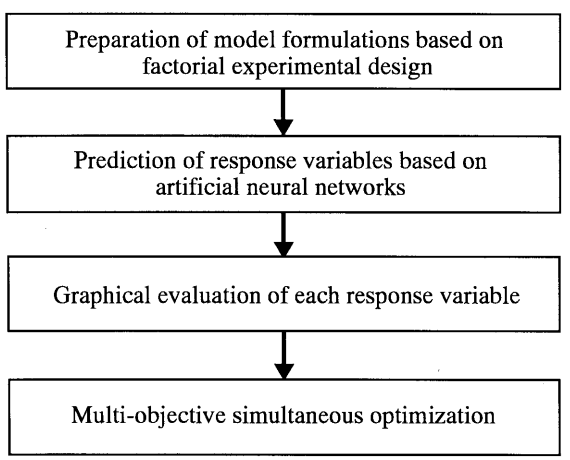

Fig. 1. Flow of the multi-objective simultaneous optimization technique incorporating ANN.

region. The aim of this study was to apply a novel simultaneous optimization technique incorporating ANN to a design of a ketoprofen hydrogel containing MET as an absorption enhancer.

2. Materials

MET was synthesized by the method described by Leffler and Calkins (1955) and was characterized by elemental analysis, nuclear magnetic resonance (NMR) spectroscopy (Jeol GSX 270F, Tokyo, Japan) and gas chromatography (GC) (Shimadzu GC-7A, Kyoto, Japan). The purity of this compound was over 99%. Ketoprofen was purchased from Sigma (St. Louis, MO). Carboxyvinylpolymer, marketed as Hiviswako 105®, was generously supplied by Wako Pure Chemical Industries (Osaka, Japan). The other chemicals used were of reagent grade.

3. Methods

3.1. Preparation of the hydrogel

As model formulations, 12 kinds of ketoprofen hydrogels composed of carboxyvinylpolymer, triethanolamine, ethanol, MET and water were prepared according to 2-factor spherical second-order composite experimental design (Khuri and Cornell, 1987) (Table 1). The amounts of ethanol (X_1) and MET (X_2) were selected as causal factors. The amounts of ketoprofen, carboxyvinylpolymer and triethanolamine were fixed at 0.30, 0.15 and 0.20 g, respectively. The total amount of each hydrogel was adjusted to 10.0 g by the addition of water.

Ketoprofen was dissolved in ethanol containing MET. Separately, carboxyvinylpolymer and triethanolamine were dissolved in water. Both components were then mixed well and the resulting hydrogel was stored at room temperature for 24 h under air-tight conditions prior to use.

3.2. Percutaneous absorption study

Male Wistar rats weighing 180–200 g were anesthetized with a carbamic acid ethyl ester solution (25%, 3 ml/kg intraperitoneally (i.p.)) and secured on their backs. Their abdominal hair was gently removed with an electric clipper. A glass cell with a 16 mm inner diameter and 10 mm in height, was attached on the shaved abdominal skin with a cyanoacrylate type of adhesive (Aron Alpha A®, Tokyo, Japan) and filled with the test hydrogel (1 ml) under occlusive conditions. Blood samples (300 μl) were taken via the jugular vein 1, 2, 4, 6 and 8 h after application. Each blood sample was centrifuged (13 000 rpm, 3 min) and the plasma sample (100 μl) was thoroughly mixed with methanol (300 μl) containing an appropriate amount of *p*-hydroxybenzoic acid *n*-butyl ester as an internal standard. The mixture was centrifuged again (13 000 rpm, 3 min) to precipitate the denatured proteins. The sample was filtered through a disposable filter unit (Sample prep® LCR4(T)-LG, Japan Millipore, Yonezawa, Japan). The concentration of ketoprofen in the filtrate was analyzed using a high-performance liquid chromatography (HPLC) system (Shimadzu, LC-5A, Kyoto, Japan) equipped with a variable wavelength ultraviolet monitor (Shimadzu, SPD-6A). The flow rate was 1 ml/min and elution was carried out at room temperature. The other analytical conditions were as follows: column, STR ODS-II, 150 × 4.6 mm

Table 1
Experimental design and model formulae of ketoprofen hydrogels

Formulation	X_1	Ethanol (%)	X_2	MET (%)
1	$\sqrt{2}$	50.0	0	1.50
2	$-\sqrt{2}$	20.0	0	1.50
3	0	35.0	$\sqrt{2}$	3.00
4	0	35.0	$-\sqrt{2}$	0
5	1	45.6	1	2.56
6	1	45.6	−1	0.44
7	−1	24.4	1	2.56
8	−1	24.4	−1	0.44
9	0	35.0	0	1.50
10	0	35.0	0	1.50
11	0	35.0	0	1.50
12	0	35.0	0	1.50

The amounts of ketoprofen, carboxyvinyl polymer and triethanolamine were fixed at 0.30, 0.15 and 0.20 g, respectively. The total amount of each hydrogel was adjusted to 10.0 g by the addition of water.

i.d. (Shimadzu); ultraviolet detection, 254 nm; mobile phase, 0.057% phosphoric acid/methanol (35:65).

3.3. Evaluation of skin irritation

Irritation evoked by model formulae on rat skin was microscopically judged after the end of experiments on percutaneous absorption. The site of application of each formula on the skin was excised from rats. The separated skins were fixed in 10% neutral carbonate-buffered formalin for at least 24 h before routine processing and then cut vertically against the skin surface at the central region at a width of 4 mm. Each section was dehydrated using a graded series of ethanol solutions and embedded in paraffin wax. Tissues were divided into small pieces (≈ 3 μm in thickness) and stained with hematoxylin and eosin. All sections were examined by light microscopy (Optiphoto, Nikon, Tokyo, Japan).

3.4. Computer programs

Computation was carried out on a desktop computer (PC-9801 BX4, NEC, Tokyo, Japan). The curve fitting program MULTI, made by Yamaoka et al. (1981), was used to estimate the pharmacokinetic parameters. The computer program ANNOP, written by us, was used for the simultaneous optimization for multi-objective problems in which ANN was incorporated (Takahara et al., 1997). The part of ANN which was based on an extended Kalman filter algorithm was written with reference to a program made by Murase et al. (1994).

4. Results and discussion

4.1. Percutaneous absorption in vivo in rats

In order to evaluate the percutaneous absorption in vivo in rats, the rate of penetration (R_p) of ketoprofen was estimated from a simple pharmacokinetic model based on the assumption that the rate of penetration of ketoprofen absorbed from the hydrogel is constant after a lag time according to the following equation (Takayama and Nagai, 1991):

$$C = \frac{R_p}{V_d k_{10}} \left\{ 1 + \frac{\beta - k_{10}}{\alpha - \beta} e^{-\alpha(t - t_L)} + \frac{k_{10} - \alpha}{\alpha - \beta} e^{-\beta(t - t_L)} \right\} \quad (2)$$

where C is the plasma concentration, R_p is the rate of penetration, t is time, t_L is the lag time, V_d is the distribution volume of the central compartment, k_{10} is the elimination rate constant from the central compartment and α and β are the hybrid first-order rate constants. The mean values of V_d, k_{10}, α and β, estimated previously, were used in this study to determine R_p and t_L values (Takayama and Nagai, 1991). Results are summarized in Table 2. A wide variation in these parameters among the experiments was observed. It was indicated that they were greatly affected by changes in the levels of causal factors such as ethanol and MET. Consequently, they were selected as response variables for seeking the optimal formulation of the ketoprofen hydrogel.

4.2. Skin damage

Skin irritation 8 h after application of ketoprofen hydrogels was pathologically investigated. The microscopic findings were graded in five levels of irritation, from no change (level 0) to a marked one (level 4) according to epidermis liquefaction

Table 2
Experimental values of response variables

Formulation	R_p (mg/h)	t_L (h)	TIS
1	1.45	0.900	17
2	0.468	0.626	4
3	1.84	0.190	16
4	0.00499	1.08	0
5	1.36	0.854	16
6	0.422	0.931	14
7	1.56	0.235	11
8	0.273	0.956	1
9	0.918	0.904	12
10	1.06	0.954	13
11	1.37	0.913	17
12	1.08	0.929	17

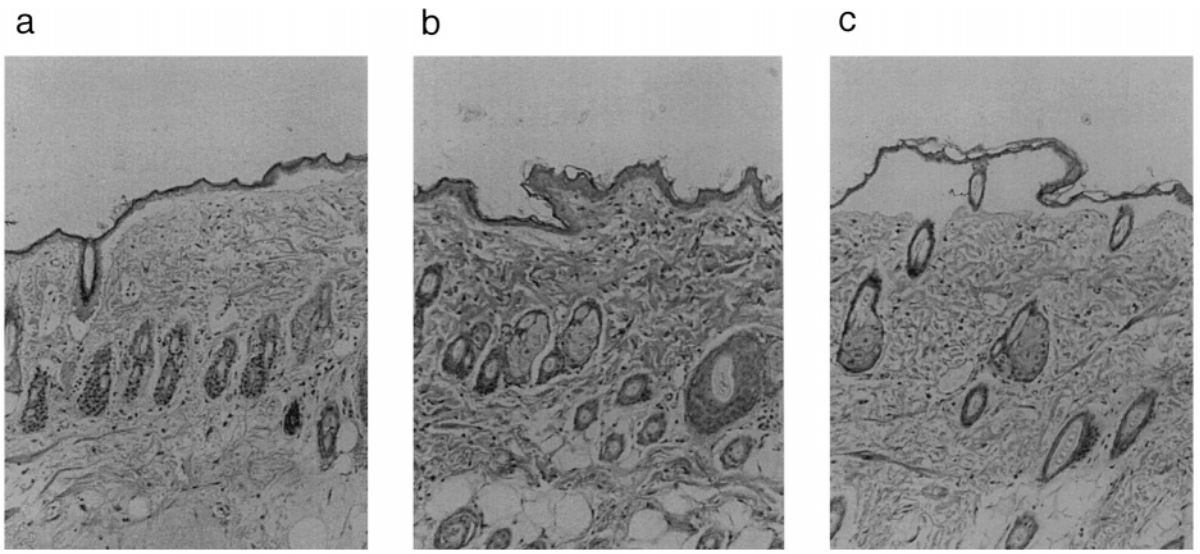

Fig. 2. Microphotographs of vertical section of rat skin 8 h after application of ketoprofen hydrogels containing MET. H & E stein; × 100; (a) formula 3; (b) formula 4; and (c) formula 9.

and collagen fiber swelling in the dermis and hypodermis, together with edema and inflammatory cell infiltration, as well as degeneration of skin appendages. Fig. 2 shows microscopic photos of rat skin 8 h after application of the model formulae (formulae 3, 4 and 9 in Table 1). Although the hydrogel not containing MET caused no change in any tissues, all the other hydrogels containing MET induced epidermis liquefaction. Further, the more MET the hydrogels contained, the more skin damage they caused. A total irritation score (TIS) was obtained by summation of each irritation score and used as an index of skin damage caused by the application of ketoprofen hydrogels. Results are summarized in Table 2. It was indicated that TIS was greatly affected by changes in the levels of causal factors. Consequently, this was selected as a response variable.

4.3. Graphical evaluation

Two causal factors corresponding to different levels of ethanol and MET were used as each unit of the input layer. The output layer was composed of three response variables, i.e. R_p, t_L and TIS, respectively. A set of causal factors and response variables was used as tutorial data for ANN and fed into a computer. Figs. 3 and 4 show the three-dimensional diagrams of each response variable as a function of X_1 (amounts of ethanol) and X_2 (amounts of MET). Nonlinear relationship between the causal factors and the response variables were represented with the response surface predicted by ANN (Fig. 3). On the other hand, PNE exhibited relatively plain surfaces for all responses (Fig. 4). Further, PNE predicted negative values in the boundary region of the experimental limits. They were out of physical reality as can be seen in Fig. 4. Generally, the quantitative relationships between causal factors and response variables in vivo are thought to be complex and nonlinear. It was suggested that ANN was more useful than PNE in cases when approximations of such relationships were required.

4.4. Simultaneous optimization

Optimization of a ketoprofen hydrogel was performed according to the generalized distance function defined in Eq. (1) under the restriction of the experimental region. The optimal values of individual response variables, $FD_i(\boldsymbol{X})$, were calculated before simultaneous optimization was car-

ried out; i.e. the individual maximum R_p, the minimum t_L and the minimum TIS values, respectively. The simultaneous optimal solutions estimated by ANN as a function of training times and the number of units in a hidden layer are given in Tables 3 and 4. These solutions were

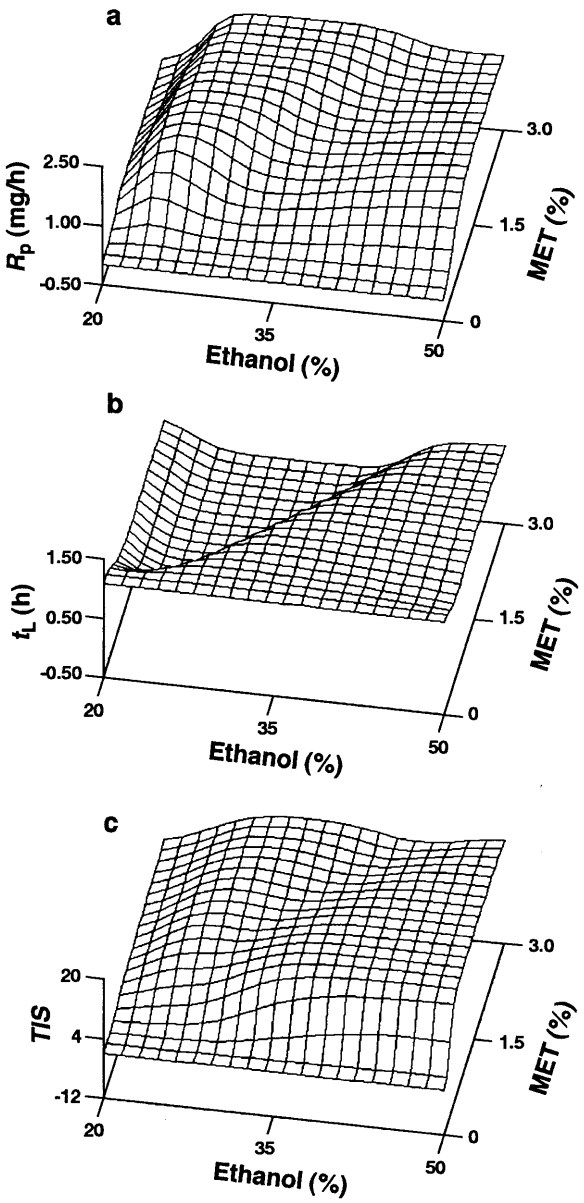

Fig. 3. Response surfaces of R_p, t_L and TIS predicted by ANN as a function of the amounts of ethanol and MET. (a) R_p; (b) t_L; and (c) TIS.

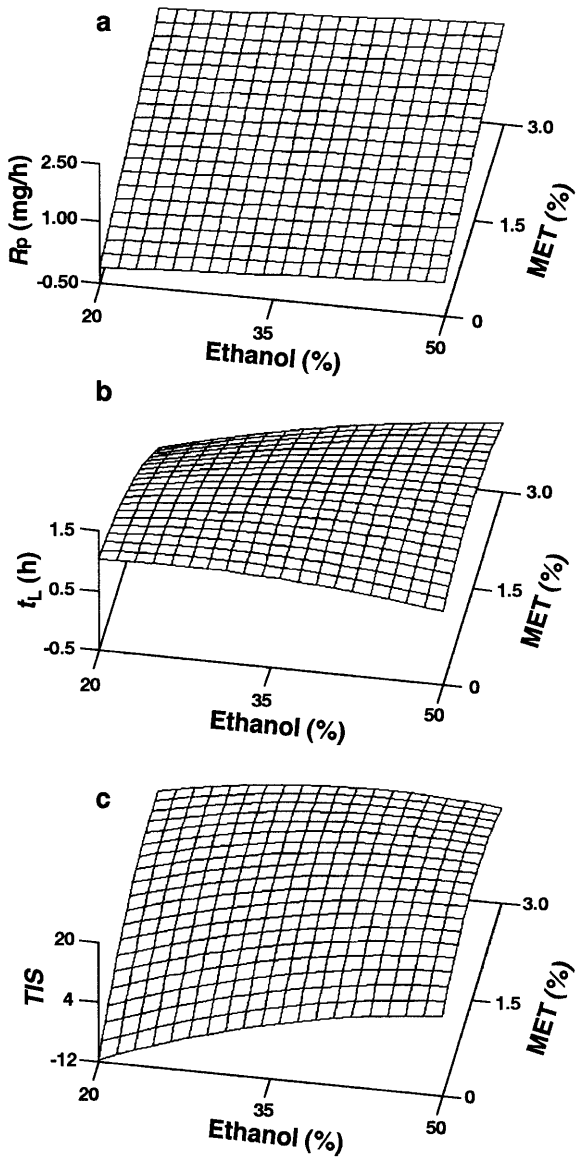

Fig. 4. Response surfaces of R_p, t_L and TIS predicted by PNE as a function of the amounts of ethanol and MET. (a) R_p; (b) t_L; and (c) TIS.

nearly stable irrespective of changes in ANN structure and training times. Thus, the average values were adopted as the optimal values (23.4% as ethanol and 2.21% as MET, respectively). The predicted and the experimental response variables for the optimal formula are given in Table 5. The observed results of R_p and TIS coincided well

Table 3
Simultaneous optimal values of X_1 (ethanol, %) estimated as a function of training times and the number of units in a hidden layer

Training times	Number of units in a hidden layer				
	3	4	5	6	7
100	23.2	22.8	22.8	23.1	23.3
200	22.0	22.8	23.4	23.4	23.2
300	23.0	22.8	24.3	23.3	23.3
400	23.5	22.9	24.5	23.4	23.5
500	23.5	22.9	24.4	23.6	23.2
600	23.4	23.5	24.5	23.1	23.5
700	23.4	23.5	24.6	23.7	23.5
800	21.8	21.5	24.5	22.7	23.2
900	23.4	23.5	24.8	23.8	23.6
1000	23.4	21.5	24.0	24.0	23.5

with the predictions although the result of t_L did not. The difference between the predicted and the experimental t_L value was ≈ 30 min. In order to predict t_L more precisely, other experiments such as an in vitro permeation study are required. However, R_p and TIS, which had a lot of influence on the effectiveness and safety, were satisfactorily predicted. We suggested that the multi-objective simultaneous optimization technique incorporating ANN was quite useful for optimizing pharmaceutical formulae when predictions of pharmaceutical responses based on PNE were limited to low levels.

Table 4
Simultaneous optimal values of X_2 (MET, %) estimated as a function of training times and the number of units in a hidden layer

Training times	Number of units in a hidden layer				
	3	4	5	6	7
100	2.19	2.37	2.38	2.41	2.19
200	2.24	2.38	2.46	2.18	2.15
300	2.40	2.38	2.15	2.19	2.24
400	2.18	2.38	2.14	2.18	2.18
500	2.18	2.39	2.15	2.18	2.40
600	2.18	2.18	2.14	2.04	2.18
700	2.18	2.18	2.14	2.17	2.18
800	2.21	2.16	2.14	2.11	2.10
900	2.18	2.18	2.13	2.17	2.18
1000	2.18	2.15	2.16	2.16	2.18

Table 5
Predicted and experimental response variables for the optimal formula

Reponse	Predicted	Experimental[a]
R_p (mg/h)	1.45	1.21 ± 0.19
t_L (h)	0.264	0.713 ± 0.106
TIS	9.42	10.8 ± 1.0

[a] The mean $\pm$ S.D. of four determinations.

Acknowledgements

The authors are very grateful to Professor Hiroshi Ichikawa, Department of Information Science, Hoshi University, for his support and valuable advice in ANN and to Professor Kimio Higashiyama, Department of Medicinal Chemistry, Hoshi University, for his help in synthesizing MET. The authors also thank Ms. Emiko Miyazawa for her kind assistance in the experimental work.

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