(2*E*,4*E*)-*N*-(4-(1*H*-Indol-3-yl)piperidin-1-yl)alkyl-5-(substituted phenyl)-2,4-pentadienamides as Antiallergic Agents with Antihistaminic and Anti Slow-Reacting Substance (SRS) Activities

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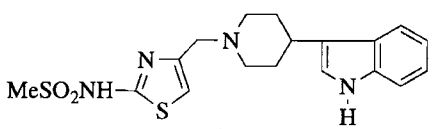
Key Words: *antianaphylactic activity; anti-SRS activity; 4-(1H-indol-3-yl)piperidine; structure-activity relationship; glucuronic acid conjugate*

Summary

As an extension of our study aiming to discover a novel compound with dual activities against histamine and slow-reacting substance (SRS), we synthesized two types of indolylpiperidine derivatives, 3 and 4–20. Testing for *in vivo* antianaphylactic activity and for *in vitro* anti-SRS activity revealed that (2*E*,4*E*)-5-(3,5-dimethoxy-4-hydroxyphenyl)-*N*-(2-(4-(1*H*-indol-3-yl)piperidin-1-yl)ethyl)-2,4-pentadienamide (11) exhibited potent dual activities with ED_{50} = 0.89 mg/kg and IC_{50} = 1.43 μM, respectively. However, the plasma concentration of unchanged 11 was very low when administered orally in guinea pigs. This result can be explained by fast formation of a glucuronic acid conjugate.

Introduction

We previously reported that the thiazole derivatives having an indolylpiperidine implied potential clinical applications to allergic diseases such as asthma, rhinitis and urticaria. For instance, *N*-(4-(4-(1*H*-indol-3-yl)piperidin-1-yl)methylthiazol-2-yl)methanesulfonamide (**1**) as illustrated in Figure 1 potently inhibited egg albumin-induced systemic anaphylaxis in guinea pigs, which is attributed mainly to antihistaminic activity, without central nervous system side effects[1]. The other thiazole derivative having an indolylpiperidine, *N*-(4-(4-(1*H*-indol-3-yl)piperidin-1-yl)methylthiazol-2-yl)-propanamide (**2**), exhibited dual activities against histamine and slow-reacting substance (SRS)[2].



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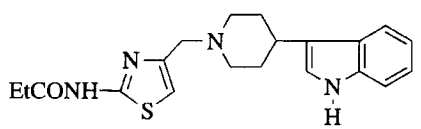

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Figure 1. Structures of the thiazole derivatives having an indolylpiperidine unit.

To extend our study on indolylpiperidine derivatives having dual activities against histamine and SRS, we next focused on the caffeic acid derivatives, TMK992[3] and TMK 777[4] (Scheme 1). These compounds have been indicated to possess inhibitory activities against histamine as well as 5-lipoxygenase (5-LO) which contributes to produce SRS. The antihistaminic activity of these compounds was assumed to be elicited by the diphenylmethylpiperazine or diphenylmethoxypiperizine moiety, and inhibition of 5-LO was due to the phenol moiety, based on the result that caffeic acid *per se* inhibited only 5-LO.[5] Freter *et al.*[6] demonstrated and we also reported in our previous paper[1] that the indolylpiperidine structure can be utilized for a surrogate of the diphenylmethylpiperazine in terms of antihistaminic activity. Therefore we planned to investigate whether the compounds in Scheme 1 designed by replacement of the diphenylmethylpiperazine moiety of TMK992 and the diphenylmethoxypiperidine part of TMK777 with the indolylpiperidine structure exhibit dual activities against histamine and SRS.

We describe herein the synthesis and structure-activity relationships of (2*E*,4*E*)-*N*-(4-(1*H*-indol-3-yl)piperidin-1-yl)alkyl-5-(substituted phenyl)-2,4-pentadienamides (**4–20**), along with the synthesis and activity of (2*E*)-3-(4-hydroxy-3-methoxyphenyl)-*N*-(2-(4-(1*H*-indol-3-yl)piperidin-1-yl)-ethyl)-2-propenamide (**3**).

Results and Discussion

Synthesis

The objective compounds were prepared as depicted in Scheme 2. The acids **21** and **22a–i**, whose phenolic hydroxy is protected with a (2-methoxyethoxy)methyl (MEM) group, were condensed with (4-(1*H*-indol-3yl)piperidin-1-yl)alkylamine **24a–c** in the presence of triethylamine using diphenylphosphinic chloride as a condensing agent[7]. Deprotection of the MEM group was accomplished by treatment with *p*-toluenesulfonic acid in methanol. Di- and trimethoxyphenylpentadienoic acid **23a,b** were condensed directly with **24a**. The compounds **17–20** were obtained by acylation of the phenolic derivatives **4** and **11** with appropriate acid chlorides in the presence of triethylamine.

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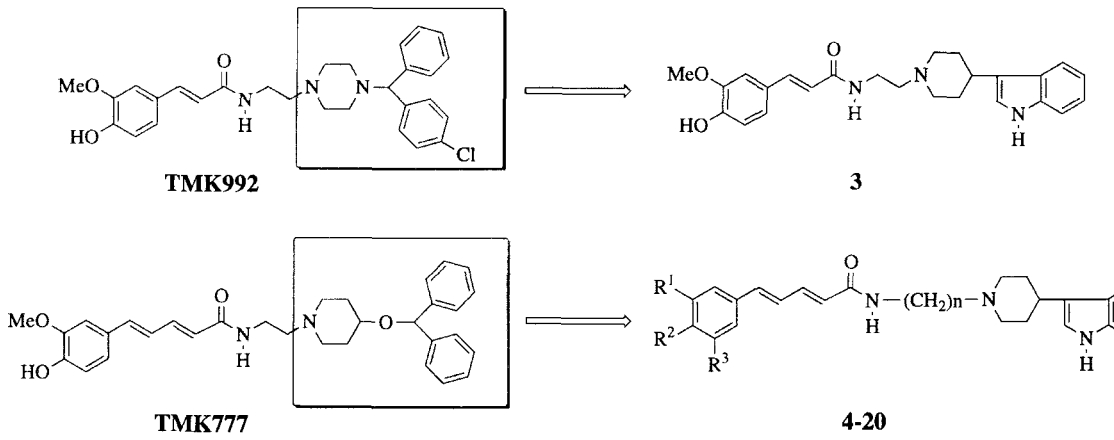
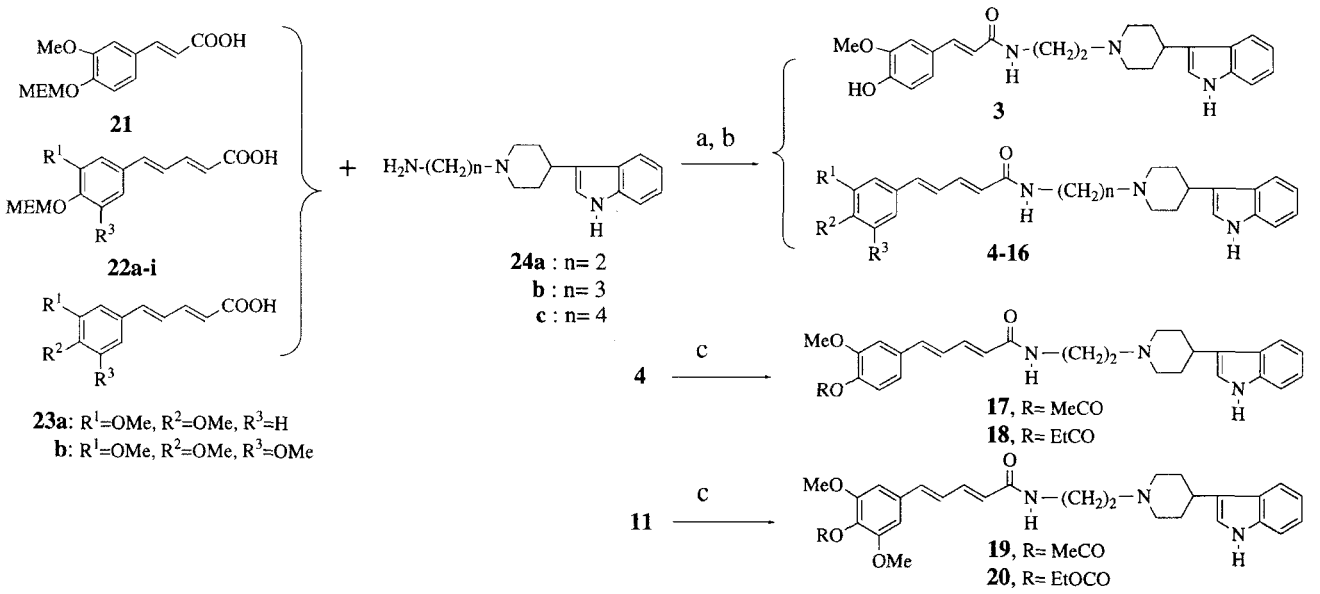
Scheme 1



Scheme 2 [a]

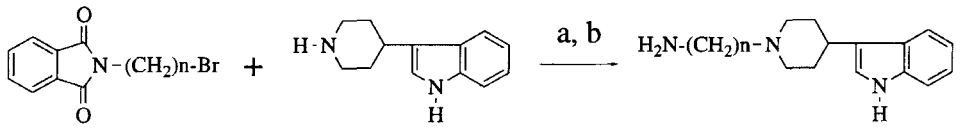


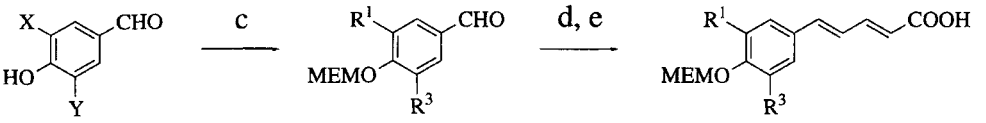
[a] (a) $(Ph)_2POCl/Et_3N$; (b) *p*-$MePhSO_3H$/MeOH, this procedure is unnecessary in **10** and **16**; (c) MeCOCl, EtCOCl, or $EtCOCl/Et_3N$.

Stereochemistry of the ene or diene moiety of the objective compounds, **3** and **4–20**, was presumed to be all *E* configurations through interpretation of the proton NMR spectra. Regarding compound **3**, only one olefin proton was assignable at 6.50 ppm as a doublet with 15.0 Hz of coupling constant (*J*); however, its intermediate protected with a MEM group had two doublets at 6.35 and 7.57 ppm each with $J = 15.0$ Hz. Of compounds **4–20**, the citric acid salt (1:1) of **11** was investigated in detail as representative. The protons of the diene moiety can be assigned as shown in Figure 2, and the *J* values are 14.7 and 15.2 Hz, indicating both as *E* configuration. Although the other compounds were not investigated in detail, one proton (the other three are overlaid by aromatic protons) is observed around 6.1 ppm as a doublet with $J = 15.0$ Hz, suggesting that the diene moiety of these compounds is also both *E* configurations.

As illustrated in Scheme 3, the (4-(1*H*-indol-3yl)piperidin-1-yl)alkylamines **24** were prepared by alkylation of the indolylpiperidine **26** with *N*-(ω-bromoalkyl)phthalimides **25** in the presence of sodium hydrogencarbonate, followed by treatment with hydrazine monohydrate in ethanol. The acids **22a–i** were prepared according to the following method; 4-hydroxybenzaldehydes **27a–i** were treated with MEM chloride and *N,N*-diisopropylethylamine, subsequently the

Scheme 3 [a]





27a; X=OMe, Y=H
b; X=Me, Y=H
c; X=Cl, Y=H
d; X=OH, Y=H
e; X=Y=OMe
f; X=Y=Me
g; X=Y=isoPr
h; X=Y=*tert*Bu
i; X=Y=Cl

28a; R^1=OMe, R^3=H
b; R^1=Me, R^3=H
c; R^1=Cl, R^3=H
d; R^1=OMEM, R^3=H
e; R^1=R^3=OMe
f; R^1=R^3=Me
g; R^1=R^3=isoPr
h; R^1=R^3=*tert*Bu
i; R^1=R^3=Cl

22a-i

[a] (a) $NaHCO_3$/DMF; (b) $NH_2NH_2 \cdot H_2O$/EtOH; (c) MEMCl/$(isoPr)_2EtN$; (d) $(EtO)_2P(O)CH_2CH=CHCOOEt$/NaH; (e) NaOH/MeOH.

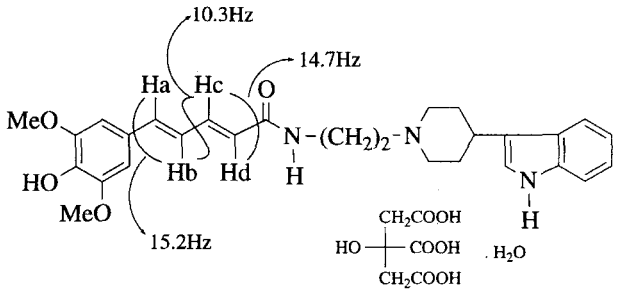


Figure 2. Coupling constants of the olefin protons in the citric acid salt (1:1) of **11**. Chemical shifts (δ, ppm) of the corresponding protons are as follows: Ha, 6.86; Hb, 7.00; Hc, 7.25; Hd, 6.13.

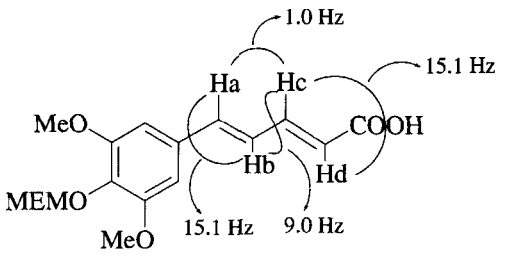


Figure 3. Coupling constants of the olefin protons in **22e**. Chemical shifts (δ, ppm) of the corresponding protons are as follows: Ha, 6.90; Hb, 6.80; Hc, 7.50; Hd, 6.10.

protected benzaldehydes **28a–i** were condensed with ethyl 4-(diethylphosphono)crotonate in the presence of sodium hydride (Wadsworth-Emmons reaction), followed by alkaline hydrolysis. The acids **23a,b** (Scheme 2) were also synthesized according to the above mentioned method using the commercially available benzaldehydes. Although the Wadsworth-Emmons reaction is well documented to generate *E,E*-diene structure[8], we confirmed by the proton-decoupling method that the stereochemistry of the diene moiety of **22e** is *E* for both the olefins (Figure 3).

Structure-Activity Relationships

Compounds **3** and **4–20** were tested for inhibitory activities against egg albumin-induced systemic anaphylaxis in guinea pigs (*in vivo* assay) and also against the synthesis or release of SRS by calcium ionophore (A23187) in rat neutrophils (*in vitro* assay). In the systemic anaphylaxis assay, each compound was administered orally 30 min prior to the antigen challenge, and protection from anaphylatic dyspnea was assessed in terms of the survival ratio[11]. The pharmacological results are shown in Table 1.

Table 1. Antianaphylactic (*in vivo*) and anti-SRS (*in vitro*) activities of compounds **3–20**.

	R^1	R^2	R^3	*n*	antianaphylactic activity[a] 10 (mg/kg, *p.o.*)	1.0	0.1	anti-SRS activity[b] IC_{50} = μM
3					3/3	0/3	0/3	33.1
4	OMe	OH	H	2	3/3	1/3	0/3	2.05
5	OMe	OH	H	3	3/3	0/3	1/3	3.48
6	OMe	OH	H	4	3/3	2/3	0/3	4.65
7	Cl	OH	H	2	3/3	1/3	0/3	102
8	Me	OH	H	2	2/3	0/3		12.1
9	OH	OH	H	2	2/3	0/3		4.41
10	OMe	OMe	H	2	0/3			76.3
11	OMe	OH	OMe	2	3/3	2/3	0/3	1.43
12	Cl	OH	Cl	2	3/3	0/3		1.34
13	Me	OH	Me	2	3/3	1/3	0/3	1.65
14	iso Pr	OH	iso Pr	2	3/3	0/3	0/3	4.00
15	*tert* Bu	OH	*tert* Bu	2	0/3			12.3
16	OMe	OMe	OMe	2	1/3			61.3
17	OMe	OCOMe	H	2	3/3	1/3	0/3	1.23
18	OMe	OCOEt	H	2	3/3	1/3	0/3	1.35
19	OMe	OCOMe	OMe	2	3/3	7/10	0/10	0.44
20	OMe	OCOOEt	OMe	2	3/3	3/3	0/3	1.32

[a] Effective number over total number of tested guinea pigs.

[b] Phenidone was used as a standard for the assay of each compound. Through the experiments (n = 18) phenidone inhibited 19.8 % ± 2.46 (SE) at 1 μg/ml and 92.3 % ± 0.95 at 10 μg/ml.

First of all, the length of the ene moiety was investigated in terms of the effects on both antianaphylactic and anti-SRS activities. The diene **4** exhibited remarkably higher anti-SRS activity compared to the corresponding ene **3**, although the potent antianaphylactic activity of these compounds was almost equal. Subsequently, we examined the effect of methylene chain length (*n*) between the piperidine and the amide parts in **4**, while keeping the diene moiety fixed. Higher antianaphylactic activity was observed with compound **6** (*n* = 4), however lengthening of the methylene chain caused a slight decrease of anti-SRS activity. We thus selected compound **4** as the target for chemical modification of substitutions on the benzene nucleus from the standpoint of balancing both activities.

Concerning the *ortho*-substituent of the phenolic hydroxy in the disubstituted compounds, an electron donating group seemed to be superior to an electron withdrawing group. Thus, compound **7** bearing a chlorine at the position resulted in remarkable decrease of anti-SRS activity, whereas a methyl (**8**) and a hydroxy (**9**) group exhibited still potent but less activity in this assay than a methoxy group (**4**). However, no significant difference in antianaphylactic activity was observed among these compounds. Methylation of the phenolic hydroxy, **10**, intriguingly resulted in a dramatic decrease in both activities. This finding is consistent with the result of caffeic acid[5] that the phenolic hydroxy at the 4 position is essential in exerting 5-LO inhibitory activity, but the great loss of antianaphylactic activity remains unclear.

With regard to the trisubstituted compounds, an additional methoxy group at the R^3 position of **4** slightly improved both activities, when **11** is compared with **4**. In contrast, the dichloro (**12**) and dimethyl (**13**) derivatives showed markedly improved anti-SRS activity in relation to their R^3 unsubstituted analogs **7** and **8**, respectively. It seems that the size of the alkyl R^1 and R^3 substituents is also important, as the following tendency was observed for **13–15**: the bigger the size of the substituent, the lower the anti-SRS activity. The methylated compound **16** also exhibited weak activities in both assays in a manner similar to **10**. As can be seen in compounds **17–20**, acylation of the phenolic hydroxy seemed to have an advantage in exerting more potent anti-SRS activity, although antianaphylactic activity was not influenced.

Pharmacological Properties of Compound 11

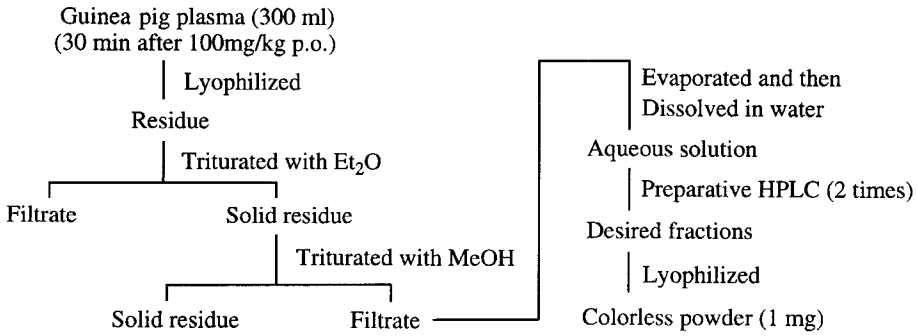
Of the compounds tested in this paper, **19** proved to be most potent in both antianaphylactic and anti-SRS activities. However, this compound seemed to easily revert to the corresponding phenolic compound **11** in the body. Hence, we selected **11** for further pharmacological evaluation. The results are summarized in Table 2, along with the data of compound **2**.

Table 2. Pharmacological properties of **2** and **11**.

Compound	Antianaphylactic activity[a] ED_{50} (mg/kg)	Anti-SRS activity[b] IC_{50} (μM)	Histamine-induced skin reaction[c] ED_{50} (mg/kg)	Histamine-induced g.p. ileum contraction[d] IC_{50} (μM)	5-LO inhibitory activity[e] IC_{50} (μM)
2	0.92	2.42	0.59	0.076	0.244
11	0.89	1.43	0.78	0.055	0.034

[a] ED_{50} values were estimated from three doses. Five aminals were used per dose.
[b] IC_{50} values were obtained from three doses. Two preparations were used per dose.
[c] ED_{50} values were estimated from four doses. Five aminals were used per dose.
[d] IC_{50} values were obtained from four doses. Five or four ileums were used per dose.
[e] IC_{50} values were obtained from three doses. Five enzyme preparations were used per dose.

Scheme 4. Isolation and purification of the major metabolite of **11** in guinea pigs.



With regard to *in vitro* antihistaminc activity, **11** inhibited the histamine-induced contraction of isolated guinea pig ileum with IC_{50} of 0.055 μM, as compared to **2** with 0.076 μM. These potent activities were reflected in the *in vivo* assays using guinea pigs. For instance, both compounds inhibited the systemic anaphylaxis induced by egg albumin and also the histamine-induced skin reaction with ED_{50} values below 1 mg/kg, when administered orally in guinea pigs. In addition, **11** indicated more potent *in vitro* activities against SRS as well as 5-LO compared to **2**. These results suggest that **11** would be useful for the treatment of antiallergic diseases where both histamine and SRS correlate.

Major Metabolite of Compound 11 in Guinea Pigs

The plasma concentration of unchanged **11** was very low at 30 min after oral administration in guinea pigs, but potent antianaphylactic activity was observed at the same time (data not shown). We thus investigated a plausible active metabolite.

Indeed, a new peak was detected on the HPLC chromatogram of the plasma which was obtained at 30 min after oral administration of **11** in guinea pigs. When the plasma was treated with β-glucuronidase[9] over 10 min at 37 °C, the metabolite reverted completely to **11**, suggesting the formation of a glucuronic acid conjugate. We next carried out isolation and purification of the metabolite according to the method shown in Scheme 4. The spectral data suggested that the diene and 2-(indolylpiperidin-1-yl)ethyl parts were not modified by metabolism; i.e., maximum absorption at 325 nm of the UV spectrum (methanol) suggested the existence of a diene group, and the fragment peaks in EI-MS at 213 (*m/z*) and 227 (*m/z*) implied 2-(indolylpiperidin-1-yl)ethylamine. We thus concluded that the conjugation with glucuronic acid occurred at the phenolic hydroxy group of **11**.

Conclusion

As an extension of our study aiming at the discovery of a novel compound with dual activities against histamine as well as SRS, we designed and synthesized two types of indolylpiperizine derivatives **3** and **4–20**. Among the compounds tested, **11** was found to have potent dual activities in the *in vitro* assays where histamine and SRS correlate respectively. However, the plasma concentration of **11** was very low, when administered orally in guinea pigs. This result can be explained by fast formation of a glucuronic acid conjugate.

Experimental

Melting points were measured on a Mitamura capillary melting point apparatus and are uncorrected. Ultraviolet (UV) spectrum was recorded on a Hitachi 320 UV spectrometer. Infrared (IR) spectra were recorded on a Shimadzu IR-408 spectrophotometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken with a Varian EM-390, a JOEL FX-270 or a

Brucker AM 400 instrument using tetramethylsilane as an internal standard. Mass spectra (MS) were obtained with a Hitachi M80 mass spectrometer (electron ionization). Elemental analyses were performed on a Perkin-Elmer 2400 CHN analyzer. Analytical results were within ± 0.4 % of the calculated values. Organic extracts were dried over anhydrous $MgSO_4$. Column chromatography was performed using Kieselgel 60 (70–230 mesh, E. Merck). The starting benzaldehydes **27f–i** were prepared by the method described in the previous papers[10].

2-(4-(1H-Indol-3-yl)piperidin-1-yl)ethylamine (**24a**)

A mixture of 4-(1*H*-indol-3-yl)piperidine (**26**)[11] (7.88 g, 39 mmol) and *N*-(2-bromoethyl)phthalimide (10.0 g, 39 mmol) and $NaHCO_3$ (3.64 g, 43 mmol) in dry *N,N*-dimethylformamide (DMF) (93 ml) was heated at 68–74 °C for 4 h. After cooling the reaction mixture was poured into ice-water (1000 ml) and the resulting precipitates were collected by filtration and washed with MeOH to afford *N*-(2-(4-(1*H*-indol-3-yl)piperidin-1-yl)ethyl)phthalimide (5.53 g, 37.6 %). An analytical sample was obtained by recrystallization from toluene as colorless crystals, mp 238–241 °C.– IR (Nujol): 3400, 3050, 1780, 1705, 1610, 1440, 1400, 1340 cm^{-1}.– ^{1}H-NMR (90MHz [D_6]DMSO): δ = 1.30–3.40 (11H, m, piperidine and ethyl protons), 3.77 (2H, d, *J* = 6.0 Hz, piperidine protons), 6.80–7.80 (5H, m, aromatic protons), 7.89 (4H, m, aromatic protons), 10.73 (1H, br. s, indole NH).– MS: *m/z* = 373 (M^+), 213.– Anal. ($C_{23}H_{23}N_3O_2$).

A mixture of *N*-(2-(4-(1*H*-indol-3-yl)piperidin-1-yl)ethyl)phthalimide (6.30 g, 16.9 mmol) and hydrazine monohydrate (2.2 g, 43.9 mmol) in EtOH (250 ml) was refluxed for 1 h. After cooling, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was triturated with cooled aqueous NaOH solution, and then the crystals formed were collected by filtration, washed with ice-water and dried in vacuo to give **24a** (4.11 g, 53.9 %) as a colorless powder, mp 98–100 °C.– IR (Nujol): 3350, 1590, 1377, 935 cm^{-1}.– ^{1}H-NMR (90MHz $CDCl_3$): δ = 1.50–3.40 (15H, m, piperidine, amine and ethyl protons), 6.70–7.70 (5H, m, aromatic protons), 8.50 (1H, br. s, indole NH).– MS: *m/z* = 243 (M^+), 213.

Compounds **24b,c** were similarly prepared by this method.

3-(4-(1H-Indol-3-yl)piperidin-1-yl)-n-propylamine (**24b**)

Compound **24b** was obtained as a colorless powder.– IR (Nujol): 3360, 3150, 1377, 1225 cm^{-1}.– ^{1}H-NMR (90MHz [D_6]DMSO): δ = 1.30–3.20 (17H, m, piperidine, amine and propyl protons), 6.70–7.70 (5H, m, aromatic protons), 10.67 (1H, br. s, indole NH).– MS: *m/z* = 257 (M^+), 213.

4-(4-(1H-Indol-3-yl)piperidin-1-yl)-n-butylamine (**24c**)

Compound **24c** was obtained as a brownish syrup.– IR (Neat): 3390, 3150, 1110, 897 cm^{-1}.– ^{1}H-NMR (90MHz [D_6]DMSO): δ = 1.00–3.20 (19H, m, piperidine, amine and butyl protons), 6.70–7.60 (5H, m, aromatic protons), 10.67 (1H, br. s, indole NH).– MS: *m/z* = 271 (M^+), 213.

(2E,4E)-5-(3,5-Dimethoxy-4-(2-methoxyethoxy)methoxyphenyl)-2,4-pentadienoic Acid (**22e**)

A mixture of 3,5-dimethoxy-4-hydroxybenzaldehyde (**27e**) (10.0 g, 55.0 mmol), *N,N*-diisopropylethylamine (8.46 g, 65.5 mmol) and 2-methoxyethoxymethyl chloride (7.47 g, 61.5 mmol) in 1,2-dichloroethane (130 ml) was refluxed for 5 h. After cooling, the reaction mixture was washed with water, dried and evaporated to give a crude syrup of **28e** (14.86 g, 100.0 %).– IR (Neat): 2950, 1690, 1590, 1500, 1460, 1420, 1390, 1330, 1230, 1120, 980 cm^{-1}.

80% Ethyl diethylphosphonocrotonate (9.20 g, 29.4 mmol) was added dropwise to a stirred suspension of 60% sodium hydride (1.41 g, 35.3 mmol) in dry tetrahydrofuran (THF) (87 ml) below 10 °C under a nitrogen atmosphere. Stirring was continued for 30 min. A solution of **28e** (7.94 g, 29.4 mmol) in THF (25 ml) was added thereto below 10 °C. After stirring for 2 h, the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in AcOEt (200 ml), the solution was washed with brine, dried and evaporated. The resulting residue was chromatographed on silica gel with a mixture of *n*-hexane and AcOEt (7:3). The desired fractions were collected and evaporated to give a powder of the ethyl ester of **22e** (7.40 g, 68.8 %).– IR (Nujol): 1700, 1620, 1580, 1500, 1425, 1350, 1330, 1305, 1250, 745 cm^{-1}.– ^{1}H-NMR (90MHz $CDCl_3$): δ = 1.22 (3H, d, *J* = 7.2 Hz, $COOCH_2Me$), 3.37 (3H, s, $OCH_2O(CH_2)_2OMe$), 3.45–3.65 (2H, m, $OCH_2O(CH_2)_2OMe$), 3.87 (3H, s, *OMe*), 3.85–4.05 (2H, m, $OCH_2O(CH_2)_2OMe$), 4.23 (2H, q, *J* = 7.2 Hz, $COOCH_2Me$), 5.27 (2H, s, $OCH_2O(CH_2)_2OMe$), 5.98 (1H, d, *J* = 15.2 Hz, CO–*CH*=CH–CH=CH, *E*), 6.70–7.50 (5H, m, CO–CH=*CH*–*CH*=*CH* and aromatic protons).– MS: *m/z* = 366 (M^+).

A mixture of the ethyl ester of **22e** (7.40 g, 20.2 mmol) and NaOH (8.0 g, 20.0 mmol) in MeOH (100 ml) was stirred for 2 h at room temperature. The reaction mixture was brought to pH 3–4 with 6N HCl under ice-cooling. The resulting crystals were collected by filtration and washed with water, diethyl ether successively to give **22e** (6.12 g, 89.6 %) as a pale yellow powder. An analytical sample was obtained by recrystallization from a mixture of AcOEt and *n*-hexane as pale yellow crystals, mp 103–104 °C.– IR (Nujol): 1720, 1620, 1580, 1500, 1425, 1350, 1300, 1205 cm^{-1}.– ^{1}H-NMR (90MHz $CDCl_3$): δ = 3.37 (3H, s, $OCH_2O(CH_2)_2OMe$), 3.50–3.70 (2H, m, $OCH_2O(CH_2)_2OMe$), 3.80 (3H, s, *OMe*), 3.85–4.05 (2H, m, $OCH_2O(CH_2)_2OMe$), 5.22 (2H, s, $OCH_2O(CH_2)_2OMe$), 5.98 (1H, d, *J* = 15.2 Hz, CO–*CH*=CH–CH=CH, *E*), 6.70–7.60 (5H, m, CO–CH=*CH*–*CH*=*CH* and aromatic protons).– MS: *m/z* = 338 (M^+). The stereochemistry of the diene moiety was confirmed by the proton-decoupling method (270 MHz) to be *E,E* (see Figure 3).

Compounds **22a–d** and **22f–i** were similarly prepared by this method. Compounds **23a,b** were also prepared from the corresponding commercially available benzaldehydes by the above mentioned condensation reaction and the following alkaline hydrolysis. The physical data are listed in Table 3.

Table 3. (2*E*,4*E*)-5-(Substituted phenyl)-2,4-pentadienoic acids.

	Yield (%)	Mp (°C)	MS (*m/z*)
22a	52.9	110–120	308, 89
22b	49.2	117–119	292, 89
22c	50.9	130–135	312, 89
22d	81.7	55–60	382, 89
22f	64.0	88–91	306, 89
22g	49.6	96–113	362, 89, 59
22h	57.7	80–90	390, 89
22i	30.3	116–120	348, 346
23a	40.0	200–205	234
23b	37.0	140–145	264

(2E)-3-(4-Hydroxy-3-methoxyphenyl)-N-(2-(4-(1H-indol-3-yl)piperidin-1-yl)ethyl)-2-propenamide (**3**)

Diphenylphosphinic chloride (1.47 g, 6.2 mmol) was added slowly to the solution of **21**[12] (1.75 g, 6.2 mmol) and triethylamine (1.81 ml, 12.9 mmol) in DMF (10 ml) at –10 – –15 °C with stirring. Stirring was continued at this temperature for 30 min, and subsequently a solution of **24a** (1.50 g, 6.2 mmol) in DMF (10 ml) was slowly added to the reaction mixture at –10 °C. After stirring for 1 h at room temperature, the reaction mixture was poured into ice-water (200 ml) and extracted with $CHCl_3$. The organic layer was washed with brine, dried and evaporated. The resulting residue was chromatographed on silica gel with a mixture of $CHCl_3$ and MeOH (10:1). The desired fractions were collected and evaporated to give the MEM-protected **3** (2.8 g, 89.2 %) as a pale yellow syrup.– IR (Neat): 1640, 1600, 1500, 1220, 745, 700 cm^{-1}.– ^{1}H-NMR (90MHz $CDCl_3$): δ = 1.60–3.30 (13H, m, piperidine and ethyl protons), 3.42 (3H, s, $OCH_2O(CH_2)_2OMe$), 3.45–3.75 (2H, m, $OCH_2O(CH_2)_2OMe$), 3.89 (3H, s, *OMe*), 3.65–3.95 (2H, m, $OCH_2O(CH_2)_2OMe$), 5.32 (2H, s, $OCH_2O(CH_2)_2OMe$), 6.35 (1H, d, *J* = 15.0 Hz, CO–*CH*=CH–, *E*), 6.52 (1H, br. s, CONH), 6.90–7.80 (8H, m, aromatic protons), 7.57 (1H, d, *J* = 15.0 Hz, CO–CH=*CH*, *E*), 8.25 (1H, br. s, indole NH).

A mixture of the MEM-protected **3** (2.0 g, 3.9 mmol) and *p*-toluenesulfonic acid monohydrate (1.05 g, 5.5 mmol) in MeOH (40 ml) was refluxed for 30 min under a nitrogen atmosphere. After removal of the solvent under

reduced pressure, the residue was diluted with water, made basic with aqueous $NaHCO_3$ solution, and extracted with AcOEt. The organic layer was washed with brine, dried and evaporated. The resulting residue was chromatographed on silica gel with a mixture of $CHCl_3$ and MeOH (10:1). The desired fractions were collected and evaporated to give **3** (0.89 g, 53.9%) as a pale yellow powder, mp 115–135 °C.– IR (Nujol): 3300, 1655, 1588, 1512 cm^{-1}.– ^{1}H-NMR (90MHz [D$_6$]DMSO): δ = 1.50–3.60 (13H, m, piperidine and ethyl protons), 3.83 (3H, s, O*Me*), 6.50 (1H, d, *J* = 15.0 Hz, CO–C*H*=CH–, *E*), 6.70–7.70 (10H, m, CO–CH=C*H* (*E*), aromatic protons and phenolic O*H*), 7.83 (1H, br. s, CONH), 10.70 (1H, s, indole NH).– MS: *m/z* = 419 (M^+), 213.– Anal. ($C_{25}H_{29}N_3O_3 \cdot 1/2\ H_2O$).

(2E,4E)-5-(3,5-Dimethoxy-4-hydroxyphenyl)-N-(2-(4-(1H-indol-3-yl)-piperidin-1-yl)ethyl)-2,4-pentadienamide (**11**)

Diphenylphosphinic chloride (0.97 g, 4.1 mmol) was slowly added to the solution of **22e** (1.35 g, 4.0 mmol) and triethylamine (1.17 ml, 8.4 mmol) in DMF (8 ml) at –10 – –15 °C with stirring. Stirring was continued at this temperature for 30 min, and then a solution of **24a** (0.97 g, 4.0 mmol) in DMF (8 ml) was slowly added to the reaction mixture at –10 °C. After stirring for 1 h at room temperature, the reaction mixture was poured into ice-water (200 ml) and extracted with $CHCl_3$. The organic layer was washed with brine, dried and evaporated. The resulting residue was chromatographed on silica gel with a mixture of $CHCl_3$ and MeOH (10:1). The desired fractions were collected and evaporated to give a pale yellow syrup which was triturated with a mixture of AcOEt and diisopropyl ether to afford a powder of the MEM-protected **11** (2.8 g, 89.2 %). IR (Nujol): 3300, 1650, 1610, 1580, 1125, 990, 960 cm^{-1}.– ^{1}H-NMR (90MHz [D$_6$]DMSO): δ = 1.60–3.50 (13H, m piperidine and ethyl protons), 3.42 (3H, s, $OCH_2O(CH_2)_2OMe$), 3.55–3.65 (2H, m, $OCH_2O(CH_2)_2OMe$), 3.80 (6H, s, O*Me*), 3.75–3.95 (2H, m, $OCH_2O(CH_2)_2OMe$), 5.03 (2H, s, $OCH_2O(CH_2)_2OMe$), 6.17 (1H, d, *J* = 15.0 Hz, CO–C*H*=CH–CH=CH–, *E*), 6.80–7.60 (10H, m, CH=C*H*–C*H*=C*H*– and aromatic protons), 8.06 (1H, br. s, CONH), 10.76 (1H, br. s, indole NH).

A mixture of the MEM-protected **11** (1.8 g, 3.2 mmol) and *p*-toluenesulfonic acid monohydrate (0.85 g, 4.5 mmol) in MeOH (36 ml) was refluxed for 30 min under a nitrogen atmosphere. The reaction mixture was worked up and purified similarly as described above for **3**. The crude crystals obtained were recrystallized from a mixture of AcOEt and EtOH (2:8) to give **11** (0.40 g, 26.3 %) as pale yellow crystals, mp 199–202 °C.– IR (Nujol): 3420, 1665, 1650, 1620, 1590, 1530, 1515, 1380, 1120 cm^{-1}.– ^{1}H-NMR (90MHz [D$_6$]DMSO): δ = 1.50–3.60 (13H, m piperidine and ethyl protons), 3.82 (6H, s, O*Me*), 6.15 (1H, d, *J* = 15.0 Hz, CO–C*H*=CH–CH=CH–, *E*), 6.80–7.70 (10H, m, CH=C*H*–C*H*=C*H*– and aromatic protons), 8.02 (1H, br. s, CONH), 8.68 (1H, br. s, OH), 10.73 (1H, br. s, indole NH). Anal. ($C_{28}H_{33}N_3O_4$).

Compounds **4–9** and **12–15** were also prepared by this method. Compounds **10** and **16** were synthesized by the reaction of **24a** with the acids **23a,b**, respectively. The physical data of these compounds are listed in Table 4.

(2E,4E)-5-(4-Acetoxy-3-methoxyphenyl)-N-(2-(4-(1H-indol-3-yl)-piperidin-1-yl)ethyl)-2,4-pentadienamide (**17**)

A solution of acetyl chloride (0.5 g, 6.3 mmol) in CH_2Cl_2 (1.0 ml) was added slowly to an ice-cooled solution of **4** (2.0 g, 4.2 mmol) and triethylamine (2.9 ml, 21.0 mmol) in DMF (20 ml) at 0 – –5 °C. After stirring for 1 h at this temperature, the reaction mixture was poured into ice-water (200 ml) and stirred for 1 h. The resulting precipitate was collected, washed with brine, and air-dried at room temperature. The precipitate was chromatographed on silica gel with a mixture of $CHCl_3$ and MeOH (20:1). The desired fractions were collected and evaporated. The residue was recrystallized from AcOEt to give **17** as a colorless powder (2.8 g, 89.2 %), mp 101–105 °C.

Compounds **18–20** were prepared similarly by this method. The physical data are listed in Table 4.

Table 4. (2*E*,4*E*)-*N*-(4-(1*H*-Indol-3-yl)piperidin-1-yl)alkyl-5-(substituted phenyl)-2,4-pentadienamides.

	R^1	R^2	R^3	*n*	Yield (%)	Mp (°C) (Recryst.solvent)[a]	Formula[b]
4	OMe	OH	H	2	33.3	115–131 (A)	$C_{27}H_{31}N_3O_3 \cdot 1/2\ H_2O$
5	OMe	OH	H	3	55.0	150–170 (A)	$C_{28}H_{33}N_3O_3 \cdot 1/2\ CHCl_3 \cdot 1/2EtOH$
6	OMe	OH	H	4	50.7	155–170 (A)	$C_{29}H_{35}N_3O_3 \cdot 1/2\ CHCl_3 \cdot 1/2EtOH$
7	Cl	OH	H	2	37.6	139–155 (B)	$C_{26}H_{28}ClN_3O_2 \cdot 4/3\ H_2O$
8	Me	OH	H	2	38.9	138–141 (B)	$C_{27}H_{31}N_3O_2 \cdot 5/4\ H_2O$
9	OH	OH	H	2	12.8	138–158 (B)	$C_{26}H_{29}N_3O_3 \cdot 6/5\ EtOH$
10	OMe	OMe	H	2	68.7	196–198 (C)	$C_{28}H_{33}N_3O_3$ [c]
12	Cl	OH	Cl	2	11.1	165–175 (E)	$C_{26}H_{27}Cl_2N_3O_2 \cdot 1/5DMF \cdot H_2O$
13	Me	OH	Me	2	23.4	125–135 (B)	$C_{28}H_{33}N_3O_2 \cdot 4/3\ H_2O$
14	iso Pr	OH	iso Pr	2	70.0	110–120 (B)	$C_{32}H_{41}N_3O_2 \cdot 6/5H_2O$
15	*tert* Bu	OH	*tert* Bu	2	43.0	108–115 (F)	$C_{34}H_{45}N_3O_2 \cdot 7/4(1,4\text{-Dioxane})$
16	OMe	OMe	OMe	2	73.5	86–100 (B)	$C_{29}H_{35}N_3O_4 \cdot 3/4\ H_2O$
17	OMe	OCOMe	H	2	22.6	101–105 (A)	$C_{29}H_{33}N_3O_4 \cdot H_2O$
18	OMe	OCOEt	H	2	25.0	157–158 (C)	$C_{30}H_{35}N_3O_4 \cdot H_2O$
19	OMe	OCOMe	OMe	2	20.7	169–172 (G)	$C_{30}H_{35}N_3O_5$
20	OMe	OCOOEt	OMe	2	53.2	90–98 (B)	$C_{31}H_{37}N_3O_6 \cdot 5/2\ H_2O$

[a] A, EtOH+$CHCl_3$; B, aqueous EtOH; C, EtOH; D, EtOH+AcOEt; E, DMF; F, 1,4-Dioxane; G, AcOEt.

[b] C, H, N, data were ± 0.4 % of the calculated values.

[c] C: calcd, 73.18; found, 73.84.

Preparation of the Citric Acid Salt (1:1) of ***11***

Compound **11** (6.0 g, 12.6 mmol) was added by small portions to a warmed solution (50 °C) of citric acid monohydrate (2.65 g, 12.6 mmol) in a mixture (50 ml) of water and EtOH (4:6) and then additional EtOH (50 ml) was added thereto. After **11** was dissolved completely, the mixture was treated with carbon powder and filtered. The filtrate was stirred for 6 h at room temperature and the resulting crystals were collected by filtration and washed with a mixture of water and EtOH to give the objective compound as pale yellow crystals (7.20 g, 85.4 %), mp 140–142 °C.– IR (Nujol): 3600, 3370, 3300, 1745, 1645, 1620, 1588, 1530, 1515, 1120 cm^{-1}.– ^{1}H-NMR (90MHz [D6]DMSO): δ = 2.40–2.80 (4H, m, citric acid methylene protons), 1.50–3.60 (13H, m, piperidine and ethyl protons), 3.80 (6H, s, O*Me*), 6.11 (1H, d, *J* = 15.0 Hz, CO–C*H*=CH–C=CH–, *E*), 6.80–7.40 (9H, m, CO–CH=C*H*–C*H*=C*H*– and aromatic protons), 7.61 (1H, d, *J* = 7.5 Hz, aromatic proton), 8.36 (1H, br. s, CONH), 8.60–9.0 (1H, br. s, phenolic OH), 10.86 (1H, s, indole NH).– MS: *m/z* = 475 (M^+), 213.– Anal. ($C_{28}H_{33}N_3O_4$). The stereochemistry of the diene moiety was confirmed by ^{1}H-NMR (270 MHz, in [D6]DMSO) to be *E,E* (see Figure 2).

Treatment of the Major Metabolite of **11** *with* β*-Glucuronidase*

Compound **11** (100 mg/kg) was administered orally to ten guinea pigs. After 30 min, whole blood was collected and centrifuged (3000 rpm) to give plasma (300 ml). The mixture of 0.1 M phosphate buffer (pH 6.5) (150 µl), β-glucuronidase (Bacterial Type 1, Sigma) (100 µl), and the plasma (50 µl) was incubated for 10 min at 37 °C. The reaction was terminated by addition of acetonitrile (200 µl). The resulting mixture was stirred for several minutes and centrifuged. The supernatant (20 µl) was injected to the HPLC. HPLC conditions: Waters 6000A; column: TSK GEL ODS (4.6 i.d. × 150 mm, 5 µm); eluent: 7 mM phosphate buffer (pH 8.0): H_2O (1:1); flow rate: 1 ml/min; detection: 330 nm. The major peak of the plasma was coeluted with **11** at 3.5 min under these conditions.

Isolation and Purification of the Major Metabolite of ***11*** *in Guinea Pigs*

The plasma (300 ml) obtained from the above mentioned procedures was lyophilized and the residue was triturated with diethyl ether and filtered. The residue was suspended with methanol (150 ml). The suspension was stirred at room temperature and filtered. After the filtrate was concentrated under reduced pressure, the resulting residue was purified by preparative HPLC. The fractions containing the objective compound were collected and lyophilized. This residue was repurified by means of preparative HPLC and lyophilized to give a colorless powder (1 mg).– ^{1}H-NMR(400 MHz CD_3OD) δ, 1.80–2.35 (6H, m), 2.63 (2H, t, *J* = 7.4 Hz), 3.1–3.8 (many peaks), 5.30–5.35 (1H, br. s), 6.15 (1H, d, *J* = 15.1 Hz), 6.8–7.1 (7H, m), 7.30–7.36 (2H, m), 7.58 (1H, d, *J* = 8.0 Hz). HPLC conditions: Shimadzu 8A system; column: AM-302 S-5 120A ODS (20 i.d. × 250 mm, 10 µm) (YMC, Kyoto, Japan); eluent: CH_3CN: H_2O (linear gradient from 1:7 to 3:7); flow rate: 10 ml/min; detection: 254 nm.

Pharmacological Methods

Antianaphylactic activity in guinea pigs[1], anti SRS-activity[2], 5-LO inhibitory activity[2] and histamine-induced skin reaction[2] were assayed as described in the previous papers.

Histamine-Induced Contraction of the Isolated Guinea Pig Ileum

Tissues were obtained from Male Hartley guinea pigs (ca. 300 g). A zigzag strip preparation of guinea pig ileums was prepared carefully and suspended in 15 ml organ bath filled with warmed (37 °C), oxygenated (95 % O_2, 5 % CO_2) standard Tyrode solution under a resting tension of 0.5 g. Tension change was monitored isometrically with a force-displacement transducer connected to a polygraph system. After 30 min equilibration period, the response to histamine (final concentration; 1×10^{-7} g/ml) was recorded 2–3 times to obtain stable contractile response, which was used as a control. After the tension of the preparation returned to basal levels by the following washing (5–6 times), histamine (1×10^{-7} g/ml) was added. The contractile responses obtained in the presence of test compounds were compared with the control.

Supplementary Material Available. Analytical data (^{1}H-NMR and IR) of compounds **22a–d, 22f–i, 23a,b, 4 – 10** and **12 – 20** are available on request.

References

[1] S. Shigenaga, T. Manabe, H. Matsuda, T. Fujii, J. Hiroi, M. Matsuo, *Chem. Pharm. Bull.* **1993**, *41* 1589–1589.

[2] S. Shigenaga, T. Manabe, H. Matsuda, T. Fujii, M. Matsuo, *Chem. Pharm. Bull.* **1994**, *42*, 1822–1827.

[3] J. Arai, Y. Koshihara, T. Wakabayashi, S. Murota, *Enssho* **1984**, *4*, 325–326. [Chem. Abstr. **1985**. *103*: 16527h].

[4] S. Ozawa, Y. Koshihara, T. Wakabayashi, S. Murota, *Enssho* **1988**, *8*, 55–58. [Chem. Abstr. **1989**. *111*: p17297k].

[5] Y. Koshihara, T. Neichi, S. Murota, A. Lao, Y. Fujimoto, T. Tatsuno, *FEBS Lett.* **1983,** *158*, 41–44 ; Y. Koshihara, T. Neichi, S. Murota, A. Lao, Y. Fujimoto, T. Tatsuno, *Biochem. Biophys. Acta.* **1984**, *792*, 92–97.

[6] K. Freter, V. Fuchs, E. Barumian, J. T. Oliver, *Arzneim.-Forsch.* **1985**, *35*, 272–276.

[7] S. Bernasconi, A. Comoni, A. Corbella, P. Gariboldi, M. Sistf, *Synthesis*, **1980**, 395–387.

[8] W. R. Roush, *J. Am. Chem. Soc.* **1978**. *100*, 3599–3601; K. Sato, S. Mizuno, M. Hirayama *J. Org. Chem.* **1967**, *32*, 177–180.

[9] M. L. Doyle, P. A. Katzman, E. A. Doisy, *J. Biol. Chem.* **1955**, *217*, 921–930.

[10] W. E. Smith, *J. Org. Chem.* **1972**, *37*, 3972–3973; H. Goot, J. C. Eriks, P. J. R. Schaar, O. P. Zuiderveld, W. T. Nauta. *Eur. J. Med. Chem.* **1978**, *13*, 425–428.

[11] J. Bergman, *J. Heterocycl. Chem.* **1970**, *7*, 1071–1076.

[12] T. Wakabayashi, Y. Kumonaka, H. Ichikawa, S. Murota (Terumo Co., Ltd.). JP 60,152,454. **1985** [Chem. Abstr. **1986**. *104*: P68618d].

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