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Structural optimization of 4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-*f*]-[1,2,4]triazolo[4,3-*a*][1,4]diazepines as antagonists for platelet activating factor: pharmacological contribution of substituents at the 2- and 6-positions of a condensed ring system

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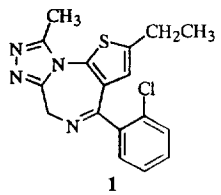
Summary — A series of 4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine derivatives bearing substituents at the 2- and 6-positions were synthesized, and evaluated in vitro for their inhibitory activity on rabbit platelet aggregation induced by platelet activating factor (PAF) and in vivo for their preventing effect on PAF-induced mortality in mice. The length of alkyl or arylalkyl side chain at the 2-position was responsible for enhancing the affinity for the PAF receptor. The simultaneous substitution at both the 2- and 6-positions resulted in a successful separation of the affinity for the PAF receptor from that for the benzodiazepine (BZ) receptor. Thus, (±)-4-(2-chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-6,9-dimethyl-6*H*-thieno[3,2-*f*][1,2,4]triazolo[4,3-*a*][1,4]diazepine (Y-24180) was confirmed to be a specific antagonist for the PAF receptor and is currently under clinical trials.

platelet activating factor (PAF) / PAF antagonist / structure–activity relationship / thienotriazolodiazepine / Y-24180

Introduction

Platelet activating factor (PAF) is generated from inflammatory cells such as macrophages in response to inflammatory and immune stimuli [1, 2], and binds to specific receptors acting as the most powerful mediator for platelet aggregation [3, 4]. Moreover, PAF possesses a variety of potent biological activities that cause bronchoconstriction, hypotension, an increase in vascular permeability, and other symptoms [5–8]. A broad scope of diseases that should be mediated by PAF has prompted us and other scientists to develop antagonistic agents for this inflammatory mediator. As a result, various research groups reported PAF antagonists with a variety of structures. Among them, triazolobenzodiazepines such as triazolam and alprazolam have been demonstrated to show an inhibitory activity for PAF-induced platelet aggregation [9]. These findings directed our attention to thieno[3,2-*f*]-[1,2,4]triazolo[4,3-*a*][1,4]diazepine as a fundamental framework in designing a structure for PAF antagonists. In our preceding paper, we reported that etizolam (**1**) and related compounds inhibited aggregation of rabbit platelet and bronchoconstriction in mice each induced by PAF [10–12]. Thus we have been involved in structural modification of **1** [13]. In contrast, some thienotriazolodiazepine derivatives, such as WEB 2086 (**2**) and E 6123 (**3**), were demonstrated to be PAF antagonists without influence on the central nervous system [14, 15].

In our preliminary study, we confirmed that **1** had a significant framework for binding to the PAF receptor. However, this compound binds to the benzodiazepine (BZ) receptor and has been used as an anxiolytic agent. Our purpose of the modification of **1** is there-



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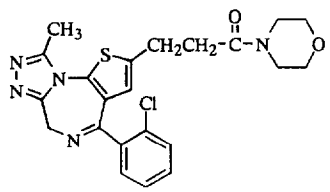

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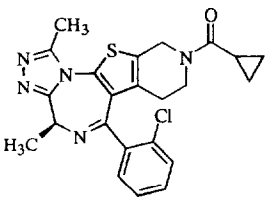

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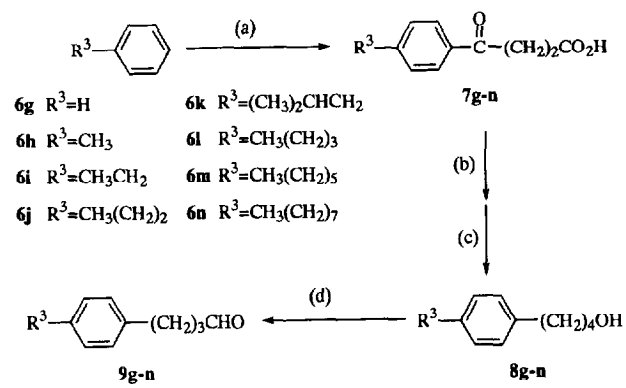
fore to enhance the antagonistic activities toward PAF and to separate the binding affinity for the PAF receptor from that for the BZ receptor. In addition, other criteria for selection are whether the PAF-antagonists are orally available and show long duration of antagonistic action. Herein we will report a structural optimization of 4-(2-chlorophenyl)-9-methyl-6*H*-thieno[3,2-*f*]-[1,2,4]triazolo[4,3-*a*][1,4]diazepine derivatives as specific and orally bioavailable PAF antagonists that fulfill our criteria.

Chemistry

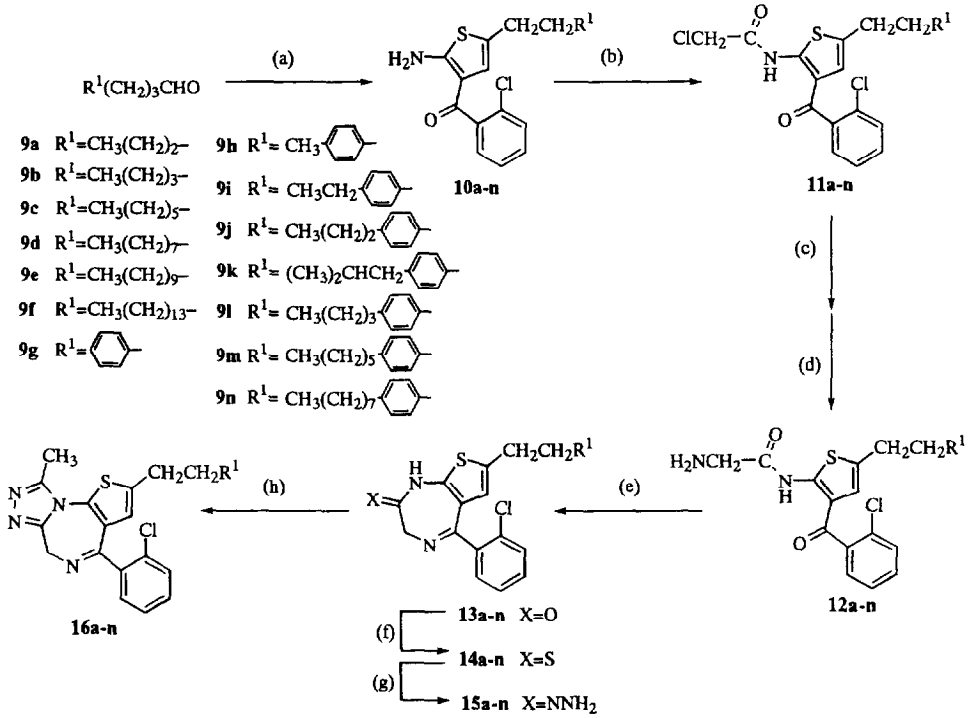
Aldehydes **9a–n** were purchased commercially or synthesized and used as practical starting materials for target compounds **16a–p**. An alternative series of aldehydes **9g–n** were synthesized. Synthetic processes for arylalkylaldehyes **9g–n** are illustrated in scheme 1. A series of 3-(4-alkylbenzoyl)propionic acids **7g–n**, which were prepared from alkylbenzenes and succinic anhydride [16], were reduced by hydrazine hydrate with potassium hydroxide and subsequently reduced by borane to give alcoholic compounds **8g–n**. The alcohols were oxidized by chromium(VI) oxide–pyridine complex (PCC) to afford **9g–n** [17]. Oxidation of



Scheme 1. (a) Succinic anhydride, $AlCl_3$; (b) $NH_2NH_2H_2O$, KOH; (c) $NaBH_4$, BF_3·OEt_2; (d) PCC, or TEMPO, NaClO.

8g–n with sodium hypochlorite in the presence of a catalytic amount of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) also proceeded smoothly to give **9g–n** [18]. This method would be suitable for a large scale synthesis of the aldehydes.

Compounds **16a–n** were prepared in the manners outlined in scheme 2. In accordance with a known method [19], each of **9a–n** was cyclocondensed with



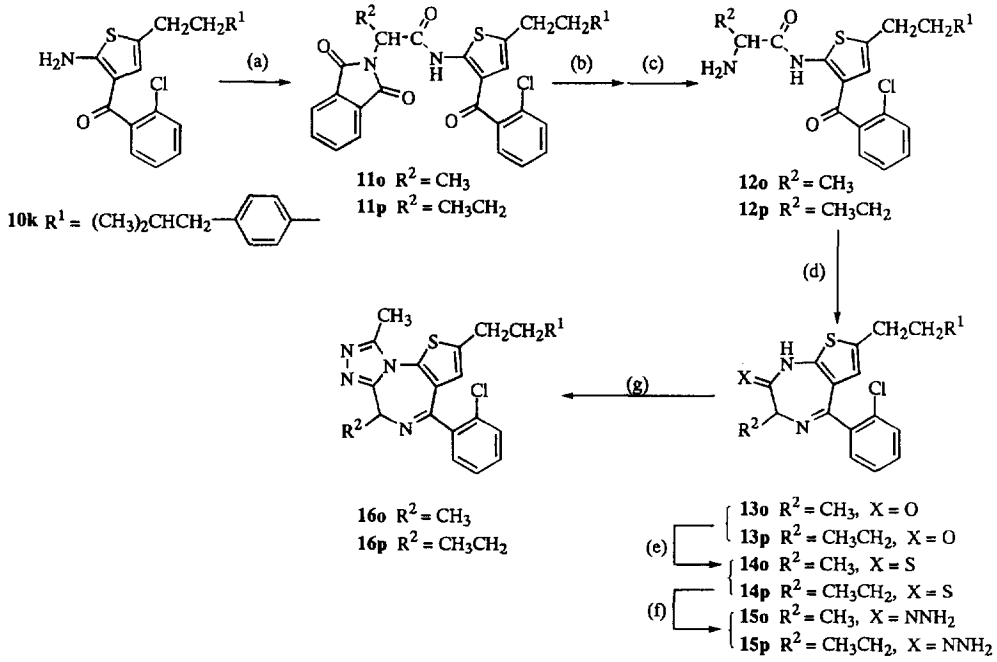
Scheme 2. (a) *o*-Chloro-ω-cyanoacetophenone, S, Et_3N; (b) $ClCH_2COCl$; (c) NaI; (d) liquid NH_3; (e) CH_3CO_2H; (f) P_2S_5; (g) NH_2NH_2·H_2O; (h) $CH_3C(OCH_2CH_3)_3$.

o-chloro-ω-cyanoacetophenone and sulfur in the presence of a catalytic amount of appropriate *tert*-amine to give 2-amino-3-benzoylthiophenes **10a–n**. In a three-step procedure, **10a–n** were converted into 2-(2-aminoacetamido)-3-benzoylthiophenes **12a–n**, which were cyclized to afford thienodiazepines **13a–n** by the method reported previously [20]. Compounds **16a–n** were stepwise derived from **13a–n** by way of **14a–n** and **15a–n** according to a published method [21].

On the other hand, 2-(2-alkyl-2-aminoacetamido)-3-benzoylthiophens **13o** and **13p** were obtained in very poor yields using the same route as for **13a–n,** possibly due to steric hindrance. For the synthesis of **16o** and **16p**, we therefore employed an alternative method (scheme 3). Thus 2-phthalimidopropionyl chloride and 2-phthalimidobutyryl chloride were reacted with **10k** to give respectively **11o** and **11p**. For the removal of the phthaloyl residue, compounds **11o** and **11p** were treated with hydrazine hydrate, followed by addition of hydrochloric acid to give **12o** and **12p**. Compounds **12o** and **12p** were then converted into **16o** and **16p** by the same procedure as for the syntheses of **16a–n**. The synthetic data for compounds **13a–p** are listed in table III, and those of compounds **16a–p** in table IV.

Results and discussion

By the method reported previously [11], compounds **16a–p** were evaluated for both their inhibitory activity against PAF-induced aggregation of rabbit platelets in vitro and their oral activity preventing against PAF-induced mortality in mice. The results are summarized in table I together with those of etizolam (**1**) and WEB2086 (**2**). We reported previously that etizolam (**1**) was a more potent PAF antagonist than triazolam [12]. This would result from the structural difference between thiophene and benzene moieties. In other words, the sulfur atom of **1** should be significant for exhibiting PAF antagonistic activities. Moreover, based on the structure–activity relationship of **1** and related compounds we tentatively concluded that the methyl group on the triazole ring of **1** would correspond to the acetoxy moiety at the 2-position of PAF. The substituents on the triazole ring of **2** were also demonstrated to fit the same pocket in PAF at the 2-position [22]. Thereby we speculated on the overlap between **1** and PAF as shown in figure 1. Here, the ethyl group at the 2-position of **1** can correspond to the alkyl chain at the 1-position of PAF. At the outset of the current study, a conventional approach to optimize a substituent at the 2-position of **1** was employed.



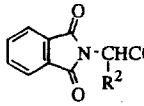
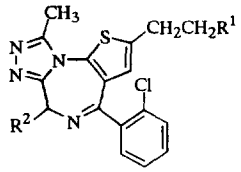
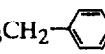
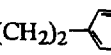
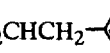
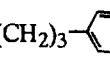
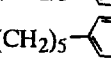
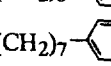
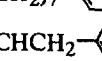
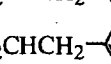
Scheme 3. (a)  (b) $NH_2NH_2 \cdot H_2O$; (c) HCl; (d) CH_3CO_2H; (e) P_2S_5; (f) $NH_2NH_2 \cdot H_2O$; (g) $CH_3C(OCH_2CH_3)_3$

Table I. Inhibitory effects of compounds **16–p** on PAF-induced rabbit platelet aggregation and mortality in mice.

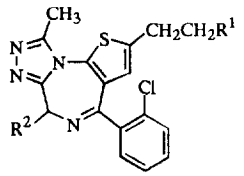


Compound	*R^1*	*R^2*	*Platelet aggregation IC_{50} (nM)*[a]		*Mortality ED_{50} (mg/kg, po)*
1 (etizolam)	H	H	6730±3250	(3)	NT[b]
16a	$CH_3(CH_2)$–	H	600	(1)	>3
16b	$CH_3(CH_2)_3$–	H	180	(1)	0.48
16c	$CH_3(CH_2)_5$–	H	280	(1)	1.0
16d	$CH_3(CH_2)_7$–	H	340	(1)	1.1
16e	$CH_3(CH_2)_9$–	H	440	(1)	>3
16f	$CH_3(CH_2)_{13}$–	H	6300	(1)	>3
16g		H	110	(1)	>3
16h		H	68	(1)	NT
16i		H	22±4.7	(3)	NT
16j		H	4.8±0.7	(3)	0.69
16k		H	4.4±1.0	(3)	0.086
16l		H	21	(1)	0.65
16m		H	4200	(1)	>3
16n		H	4200	(1)	>3
16o		CH_3–	3.8±0.7	(3)	0.021
16p		CH_3CH_2–	34	(1)	0.55
2 (WEB2086)			456±130	(3)	1.5

[a] Numbers in parentheses indicate the number of experiment. Values indicate mean ± SE when $n = 3$. [b] Not tested.

Among the compounds listed in table I, compounds **16a–f** can be regarded as simple analogues of **1**, each of which has a straight alkyl chain grafted on the terminal of the ethyl group in **1**. At first sight, both the in vitro and in vivo activities of these compounds increased with an increase in the length of the alkyl chains at the 2-position. Nevertheless, there was an optimal length which was found in **16b**, and both kinds of activity were no longer detectable for **16f**. Here the hexadecyl chain in PAF itself is just comparable to that in **16f**, but is significantly longer than the hexyl chain in **16b**. This can be explained by our speculations that the alkyl chain of PAF is folded into a hydrophobic pocket of the PAF receptor, so that this length is comparable to the length of the hexyl chain of **16b**. As shown in table II, the binding affinity for the PAF receptor was separable from that for the BZ receptor more successfully in **16b** than in **1**. However, such a separation ratio may still not be sufficient to consider **16b** for clinical trials.

Table II. Inhibitory effects of compounds **16b**, **16k**, **16o**, etizolam (**1**) and WEB2086 (**2**) on specific binding of ^{3}H-PAF to washed platelets of rabbits and ^{3}H-diazepam to synaptosomol membrane of rat brains.



Compound	*R^1*	*R^2*	*^{3}H-PAF binding IC_{50} (nM)*[a]	*^{3}H-DZP binding K_i (nM)*[a]
16b	$CH_3(CH_2)_3$–	H	1.5 [b]	177± 32
16k	$(CH_3)_2CHCH_2$–C$_6$H$_4$–	H	NT [c]	467 ± 42
16o	$(CH_3)_2CHCH_2$–C$_6$H$_4$–	CH_3–	3.5 ± 0.72	3680 ± 250
1 (Etizolam)	H	H	29.5 ± 6.2	2.34 ± 0.07
2 (WEB 2086)			9.35 ± 6.2	672 ± 44

[a] Values indicate mean ± SE of three experiments. [b] This value is mean of three experiments.
[c] Not tested.

At the next stage, in expectation of such a separation and enhancement of the activity, we therefore planned to introduce a phenyl or 4-alkylphenyl residue instead of the alkyl chain into the terminal of the ethyl group in **1** and compounds **16g–n** were synthesized as arylalkyl analogues of **16a–f**. We had already established that the introduction of a phenyl moiety to the side chain at the 2-position led to an increase in the PAF antagonistic activities in some compounds which had an arylacyloxyethyl group in place of the ethyl group of **1** [23]. So long as the alkyl chains at the 4-position of phenyl ring were straight in **16g–n**, the in vitro PAF antagonistic activity of these arylalkyl analogues increased with increasing the length of such alkyl chains, and there was also an optimal length which was found in **16j** (table I). In comparison with **16j** and **16l**, its branched-alkyl isomer **16k**, in which the number of carbon atoms in the side chain is the same as that in **16l** but the length is similar to that in **16j**, exhibited remarkably favorable activities both in vitro and in vivo, although the physicochemical properties such as lipophilicity of **16k** and **16l** were approximately equal. Thus the PAF receptor should be restrictive on the length of the side chain but tolerant of the bulkiness of the side chain.

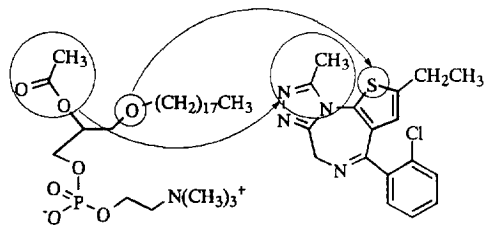
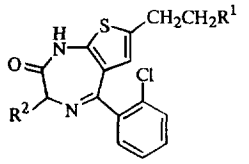


Fig 1. Overlap between PAF and **1**.

We already recognized that an alkyl substituent, especially a methyl group, at the 6-position of the condensed ring systems caused an increase in their PAF antagonistic activities, prolonged the duration of their activities, and also separated those activities from their CNS activities [24, 25]. Thus we introduced a compact alkyl substituent such as a methyl or ethyl group into the 6-position of the ring system of **16k** to give its alkyl analogues (**16o** and **16p**). Here, compound **16p** was less potent than its parent compound **16k** in both PAF antagonism in vitro and in vivo. Compound **16o** was demonstrated to be more potent than **16k** in preventing PAF-induced mortality in mice, although these two compounds showed a comparable PAF antagonistic activity in vitro (table I). In addition, **16o** exhibited practically no affinity for the BZ receptor in contrast to its potent inhibitory activity for PAF-induced aggregation (table II). This compound had previously been examined on its duration of activity by an ex vivo test. Thus the platelets that were collected from rabbits even 48 h after oral administration of **16o** (0.3 mg/kg) have been con-

Table III. Yields and physicochemical properties for compounds **13–p**.



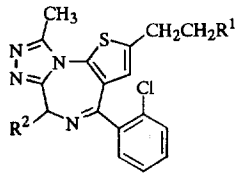
Compound	*R¹*	*R²*	*Yield*[a] *(%)*	*Mp (°C)*	*Recrystallization solvent*[b]	*Formula*
13a	CH_3CH_2–	H	18	146–148	AcOEt	$C_{17}H_{17}ClN_2OS$
13b	$CH_3(CH_2)_3$–	H	8	141–143	AcOEt	$C_{19}H_{21}ClN_2OS$
13c	$CH_3(CH_2)_5$–	H	19	131–133	AcOEt/IPE	$C_{21}H_{25}ClN_2OS$
13d	$CH_3(CH_2)_7$–	H	22	141–143	n-Hex	$C_{23}H_{29}ClN_2OS$
13e	$CH_3(CH_2)_9$–	H	8	119–121	IPE	$C_{25}H_{33}ClN_2OS$
13f	$CH_3(CH_2)_{13}$–	H	16	115–116	AcOEt/IPE	$C_{29}H_{41}ClN_2OS$
13g		H	10	175–177	AcOEt	$C_{21}H_{17}ClN_2OS$
13h	CH_3– 	H	20	195–197	AcOEt/EtOH	$C_{22}H_{19}ClN_2OS$
13i	CH_3CH_2– 	H	30	167–168	AcOEt	$C_{23}H_{21}ClN_2OS$
13j	$CH_3(CH_2)_2$– 	H	22	166–167	AcOEt	$C_{24}H_{22}ClN_2OS$
13k	$(CH_3)_2CHCH_2$– 	H	32	181–183	AcOEt	$C_{25}H_{25}ClN_2OS$
13l	$CH_3(CH_2)_3$– 	H	14	152–154	AcOEt	$C_{25}H_{25}ClN_2OS$
13m	$CH_3(CH_2)_5$– 	H	8	149–143	IPE	$C_{27}H_{29}ClN_2OS$
13n	$CH_3(CH_2)_7$– 	H	20	141–143	IPE	$C_{29}H_{33}ClN_2OS$
13o	$(CH_3)_2CHCH_2$– 	CH_3–	50	195–197	AcOEt	$C_{26}H_{27}ClN_2OS$
13p	$(CH_3)_2CHCH_2$– 	CH_3CH_2–	8	148–150	AcOEt	$C_{27}H_{29}ClN_2OS$

[a] Total yield from the corresponding aldehyde; [b] AcOEt, ethyl acetate; EtOH, Ethanol; IPE, isopropyl ether; *n*-Hex, *n*-Hexane.

firmed to show significant inhibitory activity of the aggregation by treatment with PAF [26]. Moreover, this compound can effectively protect guinea pigs from PAF-induced bronchoconstriction even 12 h after its oral administration (0.01 mg/kg), more potently than **16k** (Kagoshima et al, submitted for publication). These results with **16o** have completely fulfilled our criteria in selecting an optimal PAF antagonist. A similar contribution of such a 6-methyl substituent onto the PAF antagonistic activity and the duration of the activity has also been reported for compound **3** [15].

In conclusion, we confirmed that the modifications at both the 2- and 6-positions of the condensed ring system in **1** contributed to a significant change in the affinities for the PAF and BZ receptors. The introduction of an alkyl or arylalkyl group to the 2-position brought about a high affinity toward the PAF receptor. Moreover, the introduction of a methyl group at the 6-position of **16k** decreased the affinity for the BZ receptor without any influence on the PAF antagonistic activities. In the series of compounds synthesized, (±)-4-(2-chlorophenyl)-2-[2-(4-isobutylphenyl)-ethyl]-6,9-dimethyl-6*H*-thieno[3,2-*f*][1,2,4]tria-

Table IV. Yields and physicochemical properties for Compounds **16-p.**



Compound	*R1*	*R2*	*Yield*[a] *(%)*	*Mp (°C)*	*Recrystallization solvent*[b]	*Formula*
16a	CH_3CH_2–	H	36	107–109	IPE	$C_{19}H_{19}ClN_4S$
16b	$CH_3(CH_2)_3$–	H	17	111–113	IPE	$C_{21}H_{23}ClN_4S$
16c	$CH_3(CH_2)_5$–	H	24	80–82	IPE	$C_{23}H_{25}ClN_4S$
16d	$CH_3(CH_2)_7$–	H	43	Oil	—	—[c]
16e	$CH_3(CH_2)_9$–	H	57	64–66	PE	$C_{27}H_{35}ClN_4S$
16f	$CH_3(CH_2)_{13}$–	H	8	58–60	PE	$C_{31}H_{43}ClN_4S$
16g		H	24	106–108	AcOEt	$C_{23}H_{19}ClN_4S$
16h	CH_3– 	H	16	160–162	AcOEt	$C_{24}H_{21}ClN_4S$
16i	CH_3CH_2– 	H	23	127–128	AcOEt	$C_{25}H_{23}ClN_4S$
16j	$CH_3(CH_2)_2$– 	H	62	180–182	AcOEt	$C_{26}H_{25}ClN_4S$
16k	$(CH_3)_2CHCH_2$– 	H	39	118–121	AcOEt	$C_{27}H_{27}ClN_4S$
16l	$CH_3(CH_2)_3$– 	H	18	119–121	AcOEt	$C_{27}H_{37}ClN_4S$
16m	$CH_3(CH_2)_5$– 	H	22	136–139	AcOEt	$C_{29}H_{31}ClN_4S$ HCl 1/2H_2O
16n	$CH_3(CH_2)_7$– 	H	39	112–114	AcOEt	$C_{31}H_{35}ClN_4S$
16o	$(CH_3)_2CHCH_2$– 	CH_3–	58	129–131	AcOEt	$C_{28}H_{29}ClN_4S$
16p	$(CH_3)_2CHCH_2$– 	CH_3CH_2–	29	97–99	AcOEt/IPE	$C_{29}H_{31}ClN_4S$

[a] Total yield from the corresponding diazepine-2-one. [b] AcOEt, ethyl acetate; IPE, isopropl ether; PE, petroleum ethcr. [c] High-resolution mass spectrum was obtained with observed mass within 3 millimass units of the theoretical value.

zolo[4,3-*a*][1,4]diazepine **16o** (fig 2; Y-24180) should prove to be the most interesting compound as a PAF antagonist. On the basis of biological and toxicological results, we selected Y-24180 and it is now in clinical trials.

Experimental protocols

All melting points were measured in open capillaries and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on Jeol DS-100, JNM-EX270 and GSX-400 spectrometers and chemical shifts are expressed in ppm with tetramethylsilane (TMS) as an internal standard. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet), brs (broad singlet) and m (multiplet). Infrared (IR) spectra were recorded on a Jasco IR-810 spectrophotometer. Mass spectra (MS) were taken on Jeol JMS-OISG-

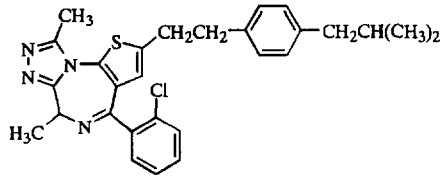


Fig 2. Structure of Y-24180.

2 and JMS-DX300 spectrometers. Elementary analyses were performed for C, H and N, and were within ± 0.4% of the calculated values. Silica-gel plates (Merck F254) and silica gel 60 (Merck, 70-230 mesh) were used for analytical and preparative column chromatography respectively.

Chemistry

4-(4-Alkylphenyl)butyraldehydes ***9g–n***
A typical example is given to represent the general procedure.

3-(4-Isobutylbenzoyl)propionic acid ***7k.*** Anhydrous aluminum chloride (175 g, 1.31 mol) was added to a suspension of succinic anhydride (52.5 g, 0.53 mol) and isobutylbenzene (52.5 g, 0.53 mol) in 1,2-dichloroethane (500 mL) with stirring below 30 °C. The reaction mixture was stirred at the same temperature for 4 h and then poured into ice and water. The organic layer was separated, washed three times with water and dried over anhydrous magnesium sulfate. After evaporation, the residue was crystallized from a mixture of ethanol and diisopropyl ether (IPE) to give **7k** (108.9 g, 93%), mp 118–120 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.9 (6H, d, J = 7.8 Hz), 1.60–2.20 (1H, m), 2.56 (2H, d, J = 7.8 Hz), 2.83 (2H, t, J = 6.4 Hz), 3.54 (2H, t, J = 6.4 Hz), 7.28 (2H, d, J = 8.2 Hz), 7.94 (2H, d, J = 8.2 Hz), 9.00–10.00 (1H, brs); IR (KBr) cm^{-1}: 1680, 1700 (C=O); MS *m/z* : 234 (M^+).

4-(4-Isobutylphenyl)butyric acid ***8k.*** Hydrazine hydrate (56 g, 1.12 mol) and potassium hydroxide (62 g, 1.12 mol) were added to a suspension of **7k** (43.5 g, 0.186 mol) in ethylene glycol (200 mL) at ambient temperature. The temperature rose to 50–60 °C. The mixture was vigorously stirred and gradually heated up to 140–160 °C till foaming ceased completely. After cooling to about 80 °C, the mixture was poured into ice and water. The aqueous solution was acidified with concentrated hydrochloric acid and extracted with toluene. The organic extracts were washed three times with water and dried over anhydrous magnesium sulfate. After evaporation in vacuo, the residue was crystallized from *n*-hexane to give **8k** (36.4 g, 89%), mp 45–47 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, d, J = 7.8 Hz), 1.60–2.10 (3H, m), 2.20–2.50 (4H, m), 2.64 (2H, t, J = 7.8 Hz), 7.05 (4H, s); IR (KBr) cm^{-1}: 1705 (C=O); MS *m/z*: 220 (M^+).

4-(4-Isobutylphenyl)butyraldehyde ***9k.*** PCC method: Chromium(VI) oxide (74 g, 0.74 mol) was added to a suspension of dry celite (150 g) and anhydrous pyridine (120 g, 1.52 mol) in methylene chloride (1500 mL). To the suspension was added dropwise a solution of **8k** (31.3 g, 0.152 mol) in methyl ene chloride (500 mL) below 20 °C and the mixture was stirred at ambient temperature for an additional 1.5 h. After filtration, the celite on the filter was washed three times with methylene chloride. The combined filtrates were washed with water, 5% hydrochloric acid and brine, successively, and dried over anhydrous magnesium sulfate. After evaporation in vacuo, the residue was distilled under reduced pressure to give **9k** (30 g, 97%), bp 122 °C / 1 mmHg; ^{1}H-NMR ($CDCl_3$) δ: 0.91 (6H, d, J = 7.8 Hz), 2.20–2.50 (4H, m), 2.63 (2H, t, J = 7.8 Hz), 7.05 (4H, s), 9.67 (1H, m); IR (KBr) cm^{-1}: 1730 (C=O); MS *m/z*: 204 (M^+).

TEMPO method: 2,2,6,6-Tetramethyl-1-piperidinyloxy (0.20 g, 0.0013 mol) was added to a solution of **8k** (50.0 g, 0.24 mol) in toluene (350 mL) and 5% aqueous sodium bicarbonate (280 mL). To the mixture was added dropwise a 10% aqueous solution of sodium hypochlorite (200 g containing 0.26 mol as sodium hypochlorite) at 0–5 °C, and the reaction mixture was stirred vigorously at the same temperature for 0.5 h. The organic layer was separated and washed with brine. The organic solution of **9k** was used for the next reaction without further purification.

The other aldehydes (**9g–j** and **9l–n**) were prepared in a similar manner.

1,3-Dihydro-2H-thieno[2,3-e]-1,4-diazepin-2-ones ***13a–n***
A typical example is given to represent the general procedure.

[2-Amino-5-[2-(4-isobutylphenyl)ethyl]-3-thienyl](2-chlorophenyl) ketone ***10k.*** Triethylamine (18.5 g, 0.18 mol) was added to a suspension of *o*-chloro-ω-cyanoacetophenone (29 g, 0.16 mol) and sulfur (4.9 g, 0.17 mol) in *N,N*-dimethylformamide (DMF) (50 mL) at ambient temperature. The aldehyde **9k** (30 g, 0.15 mol) was added to the mixture at ambient temperature; the mixture was stirred at 50–60 °C for 4 h and then poured into ice and water. The aqueous mixture was extracted twice with ethyl acetate. The combined extracts were washed with 5% hydrochloric acid, 5% aqueous sodium bicabonate, and water, and dried over anhydrous magnesium sulfate. After evaporation in vacuo, the residue was chromatographed on a silica-gel column to give **10k** (41.1 g, 70%); ^{1}H-NMR ($CDCl_3$) δ: 0.89 (6H, d, J = 7.2 Hz), 1.60–2.20 (1H, m), 2.43 (2H, d, J = 7.2 Hz), 2.79 (4H, s), 5.60–6.60 (2H, brs), 6.06 (1H, s), 7.03 (4H, s), 7.25–7.48 (4H, m); IR (liquid) cm^{-1} : 1585 (C=O), 3275, 3380 (NH); MS *m/z*: 397 (M^+).

2-Chloro-N-[3-(2-chlorobenzoyl)-5-[2-(4-isobutylphenyl)-ethyl]-2-thienylacetamide ***11k.*** Chloroacetyl chloride (12.5 g, 0.11 mol) was added to a solution of **10k** (41.1 g, 0.10 mol) in chloroform (500 mL) and refluxed for 2 h. After cooling, the reaction mixture was washed twice with 5% aqueous sodium bicarbonate and water, and dried over anhydrous magnesium sulfate. After evaporation in vacuo, the residue was chromatographed on a silica-gel column to give **11k** (45.4 g, 93%) as an oil; ^{1}H-NMR ($CDCl_3$) δ: 0.89 (6H, d, J = 7.2 Hz), 1.60–2.00 (1H, m), 2.44 (2H, d, J = 7.2 Hz), 2.80–3.00 (4H, brs), 4.31 (2H, s), 6.35 (1H, s), 7.03 (4H, s), 7.25–7.48 (4H, m), 12.50–12.80 (1H, brs); IR (neat) cm^{-1}: 1625, 1685 (C=O), 3220 (NH); MS *m/z*: 473 (M^+).

2-Amino-N-[3-(2-chlorobenzoyl)-5-[2-(4-isobutylphenyl)-ethyl]-2-thienylacetamide ***12k.*** Sodium iodide (17.3 g, 0.115 mol) was added to a solution of **11k** (45.4 g, 0.096 mol) in tetrahydrofuran (500 mL) and stirred under reflux for 2 h. After cooling, the reaction mixture was cooled in an dry ice/acetone bath. Liquid ammonia (30 mL) was added in one portion to the mixture below –30 °C and allowed to warm to room temperature within 4 h. After evaporation in vacuo, the residue was dissolved with chloroform. The organic solution was washed with water and dried over anhydrous magnesium sulfate. Removal of solvent gave **12k** (43 g) as a crude oil; ^{1}H-NMR ($CDCl_3$) δ: 0.89 (6H, d, J = 7.2 Hz), 1.60–2.00 (1H, m), 2.43 (2H, d, J = 7.2 Hz), 2.89 (4H, brs), 4.31 (2H, s), 6.35(1H, s), 7.03 (4H, s), 7.25–7.48 (4H, m); IR (neat) cm^{-1}: 1625, 1690 (C=O), 3200, 3400 (NH); MS *m/z* : 454 (M^+).

4-(2-Chlorophenyl)-7-[2-(4-isobutylphenyl)ethyl]-1,3-dihydro-2H-thieno[2,3-e]-1,4-diazepin-2-one ***13k.*** Acetic acid (8.5 g, 0.14 mol) was added to a solution of **12k** (43 g, 0.095 mol) in isopropyl alcohol (300 mL) and the mixture was refluxed for 20 h. After evaporation in vacuo, the residue was dissolved with chloroform and then the organic solution was washed with 5% aqueous sodium bicabonate and dried over anhydrous

magnesium sulfate. After removal of the solvent, the residue was crystallized from ethyl acetate to give **13k** (10 g, 49%); mp 181–183 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.89 (6H, d, J = 7.2 Hz), 1.64–2.04 (1H, m), 2.43 (2H, d, J = 7.2 Hz), 2.89 (4H, s), 4.47 (2H, s), 6.15(1H, s), 7.01 (4H, s), 7.18–7.52 (4H, m), 9.68-9.96 (1H, brs); IR (KBr) cm^{-1}: 1685 (C=O); MS *m/z*: 436 (M$^+$); anal $C_{25}H_{25}ClN_2OS$ (C, H, N).

The other compounds (**13a–n**) in table III were prepared in a similar manner.

*3-Alkyl-1,3-dihydro-2H-thieno[2,3-e]-1,4-diazepin-2-ones **13o** and **13p***

A typical example is given to represent the general procedure.

*(±)-N-[3-(2-chlorobenzoyl)-5-[2-(4-isobutylphenyl)ethyl]-2-thienyl]-2-phthalimidopropanamide **11o**.* (±)-2-Phthalimido-propanoyl chloride (23.2 g, 0.098 mol) was added to a solution of **10k** (32.5 g, 0.082 mol) in chloroform (300 mL) and the mixture was refluxed for 2 h. The reaction mixture was washed with water and 5% aqueous sodium bicarbonate and then dried over anhydrous magnesium sulfate. After evaporation in vacuo, the residue was crystallized from methanol to give **11o** (46.2 g, 94%); mp 118–120 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.87 (6H, d, J = 7.2 Hz), 1.60–2.04 (1H, m), 1.93 (2H, d, J = 7.8 Hz), 2.43 (2H, d, J = 7.2 Hz), 2.72–3.06 (4H, brs), 5.23 (1H, q, J = 7.8 Hz), 6.29(1H, s), 7.01 (4H, s), 7.16–7.48 (4H, m), 7.64–8.00 (4H, m), 11.32–11.52 (1H, brs); IR (KBr) cm^{-1}: 1625, 1705, 1720 (C=O), 3200 (NH); MS *m/z*: 598 (M+); anal $C_{34}H_{21}ClN_2O_4S$ (C, H, N).

*(±)-2-Amino-N-[3-(2-chlorobenzoyl)-5-[2-(4-isobutyl phenyl)ethyl]-2-thienyl]propionamide **12o**:.* Hydrazine hydrate (3.2 g, 0.053 mol) was added to a suspension of **11o** (22.1 g, 0.054 mol) in methanol (650 mL) and the mixture was stirred for 4 h at room temperature. Hydrochloric acid (35%; 20 mL) was added and the reaction mixture refluxed for 1.5 h. After removal of solvents, chloroform was added to the residue and the insoluble mass filtered off. The filtrate was washed with water and 5% aqueous sodium bicarbonate, then dried over anhydrous magnesium sulfate and evaporated in vacuo. The crude **12o** was used for the cyclization reaction without further purification. The analytical sample was purified with a silica-gel column; ^{1}H-NMR ($CDCl_3$) δ: 0.9 (6H, d, J = 7.2 Hz), 1.53 (3H, d, J = 7.8 Hz), 1.60–2.00 (1H, m), 1.80–2.20 (2H, brs), 2.43 (2H, d, J = 7.2 Hz), 2.66–3.00 (4H, brs), 3.82 (1H, q, J = 7.8 Hz), 6.32(1H, s), 7.02 (4H, s), 7.20–7.48 (4H, m), 12.46–13.00 (1H, brs); IR (KBr) cm^{-1}: 1610, 1695 (C=O), 3225, 3380 (NH); MS *m/z*: 468 (M$^+$).

*(±)-5-(2-Chlorophenyl)-7-[2-(4-isobutylphenyl)ethyl]-3-methyl-1,3-dihydro-2H-thieno[2,3-e]-1,4-diazepin-2-one **13o**.* Acetic acid (4.8 g, 0.08 mol) was added to a solution of **12o** prepared above in isopropyl alcohol (300 mL) and the mixture was refluxed for 20 h. After removal of the solvent, the residue was dissolved in chloroform. The organic solution was washed with 5% aqueous sodium bicarbonate and brine and then dried over anhydrous magnesium sulfate. After evaporation, the residue was crystallized from ethyl acetate to give **13o** (15.3 g, 63% from **11o**); mp 188–190 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.91 (6H, d, J = 7.2 Hz), 1.60–2.00 (1H, m), 1.78 (3H, d, J = 7.8 Hz), 2.45 (2H, d, J = 7.2 Hz), 2.72–3.08 (4H, brs), 3.94 (1H, q, J = 7.8 Hz), 6.20 (1H, s), 7.03 (4H, s), 7.22–7.72 (4H, m), 8.96–9.32 (1H, brs); IR (KBr) cm^{-1}: 1690 (C=O); MS *m/z*: 450 (M$^+$); anal $C_{26}H_{27}ClN_2OS$ (C, H, N).

The compound **13p** in table III was prepared in a similar manner.

*4-(2-Chlorophenyl)-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepines **16a–n***

A typical example is given to represent the general procedure.

*5-(2-Chlorophenyl)-7-[2-(4-isobutylphenyl)ethyl]-1,3-dihydro-2H-thieno[2,3-e]-1,4-diazepine-2-thione **14k**.* Phosphorus pentasulfide (3.3 g, 0.014 mol) was added in portions to a solution of **13k** (9.8 g, 0.022 mol) in chloroform (200 mL) and the reaction mixture was stirred under reflux for 4 h. After cooling, the mixture was washed with 5% aqueous sodium bicarbonate and dried over anhydrous magnesium sulfate. After removal of the solvent, the residue was chromatographed on a silica gel column to give **14k** (9.6 g, 94%); ^{1}H-NMR (CDCl3) δ: 0.89 (6H, d, J = 7.2 Hz), 1.68–1.96 (1H, m), 2.43 (2H, d, J = 7.2 Hz), 2.40–3.08 (4H, brs), 4.88 (2H, s), 6.17 (1H, s), 7.02 (4H, s), 7.20–7.52 (4H, m); MS *m/z*: 452 (M$^+$).

*5-(2-Chlorophenyl)-2-hydrazino-7-[2-(4-isobutylphenyl)ethyl]-3H-thieno[2,3-e]-1,4-diazepine **15k**.* Hydrazine hydrate (100%; 3.5 g, 0.070 mol) was added to a suspension of **14k** (9.6 g, 0.021 mol) in methanol (100 mL). The suspension was stirred for 1 h and the crystalline mass disappeared gradually. After removal of methanol, the residue was dissolved in chloroform and the organic solution was washed twice with water and brine and dried over anhydrous magnesium sulfate. The organic layer was evaporated in vacuo and the residue chromatographed on a silica-gel column to give **15k** (8.7 g, 91%); ^{1}H-NMR ($CDCl_3$) δ: 0.89 (6H, d, J = 7.2 Hz), 1.68–1.96 (1H, m), 2.43 (2H, d, J = 7.2 Hz), 2.88 (4H, brs), 4.16 (2H, s), 4.60–5.12 (3H, brs), 6.09 (1H, s), 7.03 (4H, s), 7.12–7.48 (4H, m); IR (KBr): 3180, 3220 (NH); MS *m/z*: 450 (M$^+$).

*4-(2-Chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-9-methyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine **16k**.* Ethyl orthoacetate (5.8 g, 0.036 mol) was added to a solution of **15k** (8.0 g, 0.021 mol) in toluene (200 mL) and the reaction mixture was refluxed for 2 h. After removal of the solvent, the residue was chromatographed on a silica-gel column to give **16k** (7.2 g, 85%) which was recrystallized from ethyl acetate; mp 118–121 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.89 (6H, d, J = 7.2 Hz), 1.64–2.05 (1H, m), 2.45 (2H, d, J = 7.2 Hz), 2.63 (3H, s), 2.78–3.24 (4H, m), 4.92 (2H, s), 6.33 (1H, s), 7.03 (4H, s), 7.25–7.52 (4H, m); MS *m/z*: 474 (M$^+$); anal $C_{27}H_{27}ClN_4S$ (C, H, N).

The other compounds (**16a–n**) in table IV were prepared in a similar manner.

*(±)-4-(2-Chlorophenyl)-2-[2-(4-isobutylphenyl)ethyl]-6,9-dimethyl-6H-thieno[3,2-f][1,2,4]triazolo[4,3-a][1,4]diazepine **16o**.* This was obtained as described for **16k** from **13o** (77%); mp 131–133 °C; ^{1}H-NMR ($CDCl_3$) δ: 0.89 (6H, d, J = 7.2 Hz), 1.60–2.00 (1H, m), 2.09 (3H, d, J = 7.8 Hz), 2.43 (2H, d, J = 7.2 Hz), 2.64 (3H, s), 2.70–3.16 (4H, brs), 4.32 (1H, q, J = 7.6 Hz), 6.34 (1H, s), 7.02 (4H, s), 7.12–7.48 (4H, m); MS *m/z*: 488 (M+); anal $C_{28}H_{29}ClN_4S$ (C, H, N).

The compound **16p** in table IV was prepared in a similar manner.

Pharmacology

PAF-induced platelet aggregation assay

Platelet aggregation studies were carried out according to the method previously described [11]. Blood samples were collected from the carotid arteries of rabbits (2–2.5 kg body weight) with siliconized syringes containing 0.1 volumes of 3.6% sodium citrate and 0.1 volumes of ACD solution (38 mM

citric acid, 38 mM sodium citrate and 138 mM glucose, pH 6.4). Platelet-rich plasma (PRP) was prepared by centrifuging the blood at 200 *g* for 10 min at 4 °C and platelet-poor plasma (PPP) was obtained by centrifuging at 1400 *g* for 10 min at 4 °C. The platelet pellets were finally resuspended in standard platelet buffer containing 0.9 mM $CaCl_2$ and 0.1% bovine serum albumin. Platelet aggregation was measured at 37 °C with a turbidimeric device (NKK Hematracer 1, model PAF-6A, Niko Bioscience, Tokyo, Japan). Inhibition of platelet aggregation was assessed by comparing the maximal change in transmitted light in test compound-treated PRP with that in vehicle-treated PRP. The aggregometer was adjusted in sensitivity to give light transmission values of 0 and 100% for PRP and PPP respectively. For each experiment, the aggregation reagent (PAF) was used at the predetermined minimal concentration which induced maximal aggregation. Inhibitory activities of the test compounds were expressed as IC_{50} values, ie, the concentrations required to inhibit platelet aggregation response by 50%.

PAF-induced mortality assay

The mortality study was carried out according to the method of Young [27]. Groups of 5–10 male ICR mice weighing 25–30 g were used. PAF (75 μg/kg) solution was administered intravenously through the lateral tail vein. All animals were observed for at least 60 min after PAF injection and the survival rate was recorded. The test compounds and the reference PAF antagonist were administered orally (0.1 mL/10 g) 1 h before the PAF injection. The ED_{50} value was calculated by the probit method as the effective doses required to prevent PAF-induced mortality by 50%.

PAF receptor binding assay

Binding of ^{3}H-PAF to platelets was performed by the previously described method [28]. A 920 μL aliquot of buffer containing 10^8 platelets was added to a siliconized tube containing 40 μL of a known concentration of test compound in a phosphate buffered saline (PBS, 137 mM NaCl, 2.6 mM KCl, 8 mM Na_2HPO_4, 0.3 mM KH_2PO_4, pH 7.1) and was preincubated at 25 °C for 5 min. The reaction was started by adding 40 μL ^{3}H-PAF (final concentration 1 nM) to the tube at 25 °C for 10 min and stopped by adding 4 mL of ice-cold saline containing 0.1% bovine serum albumin (washing solution). Platelets were isolated by vacuum filtration through Whatman GF/F filters. The filters were immediately washed four times with 4 mL ice-cold washing solution and the radioactivity was measured with a liquid scintillation counter (Beckman LS 1701). Binding in the presence of 1 mM unlabeled PAF was defined as nonspecific binding. Specific binding was defined as the difference between the total binding and nonspecific binding.

Benzodiazepine binding assay

Binding of ^{3}H-diazepam (^{3}H-DZP) to crude synaptosomal membranes of rat brains was performed as described by Möller and Okada [29]. The reaction was started by the addition of a 900-μL aliquot of crude synaptosomal membranes protein to 100-μL solution containing ^{3}H-DZP (final concentration 2 nM) and a known concentration of test compound in ethanol at 0 °C for 10 min. The binding was stopped by adding 4 mL ice-cold 50 mM Tris-HCl buffer (pH 7.4) containing 120 mL NaCl and 5 mM KCl. The samples were filtered under vacuum through Whatman GF/G filters and immediately washed four times with 4 mL ice-cold buffer (mentioned above).The radioactivity on the filters was measured with a liquid scintillation counter (Beckman LS 3081). Binding in the presence of 1 μL unlabeled DZP was defined as nonspecific binding. Specific binding was defined as the difference between the total binding and nonspecific binding.

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