4-Alkyl-1,4-dihydropyridines Derivatives as Specific PAF-Acether Antagonists

Carlos E. Sunkel,* Miguel Fau de Casa-Juana, Luis Santos, M. Mar Gômez, Mercedes Villarroya, M. Antonia González-Morales, Jaime G. Priego, and M. Pilar Ortega*

ALTER, S.A., Research Department, Mateo Inurria, 30, 28036 Madrid, Spain. *Received February 12, 1990*

A new series of 4-alkyl-1,4-dihydropyridines (1,4-DHP) were synthesized and evaluated for their ability to inhibit washed rabbit platelet aggregation induced by PAF-acether (1-*O*-hexadecyl/octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphorylcholine) and to reverse PAF-induced hypotension in anesthetized rats. Additionally, compounds were evaluated for their ability to inhibit the binding of radiolabeled PAF to its receptor on rabbit platelets. Among these compounds, **6I** and **6L** were the most potent and specific antagonists. At concentrations up to 100 μM, neither compound **6I** nor compound **6L** caused platelet aggregation nor did they inhibit platelet aggregation induced by collagen or adenosine diphosphate. Compound **6L** did not show in vitro calcium channel blocker activity measured on vascular smooth muscle preparations of rabbit aorta and on [^{3}H]nitrendipine binding assays. The compound did not show any cardiovascular effects in anesthetized rat at iv doses up to 1000 μg/kg, and the K_i value was 568.62 nmol. These results indicate that compound **6L** is a potent and specific PAF antagonist with 1,4-dihydropyridine structure but devoid of a significant cardiovascular activity related to calcium-antagonist properties.

Introduction

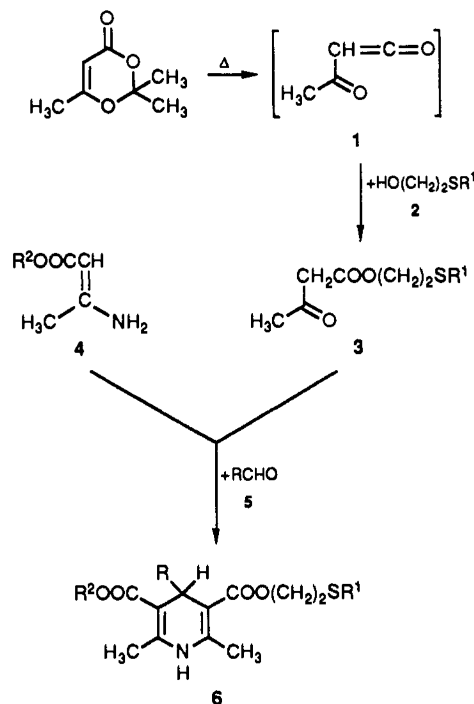
Our group has been interested for several years in the modification of the dihydropyridine ring, looking for new activities associated with this nucleus. 1,4-Dihydropyridines have been traditionally considered as cardiovascular drugs. Nevertheless, this could not be the only activity achieved by such a chemical structure. Recently, we described the synthesis and pharmacological characteristics of a new series of 1,4-DHP derivatives with enhanced antithrombotic activity which are basically free of cardiovascular effects.[1]

Going ahead in the search for new effective drugs we found a new striking fact. During the screening for structures with PAF-antagonistic activity, a series of 4-alkyl-1,4-dihydropyridines, with a thioether function at the C3-position of the dihydropyridine ring, attracted our attention. These compounds potently inhibited the PAF-induced platelet aggregation in vitro and were also active against PAF effects in vivo. They inhibited the binding of [^{3}H]PAF to rabbit platelets and were basically free of cardiovascular effects. These results encouraged us to synthesize new compounds for further evaluation.

PAF-acether (1-*O*-hexadecyl/octadecyl-2-*O*-acetyl-*sn*-glycero-3-phosphorylcholine) is a phospholipidic mediator generated in inflammatory and allergic responses.[2-3] It induces platelet and neutrophil aggregation, bronchoconstriction, and hypotension and increases vascular permeability.[4,5] The development of specific PAF antagonists is therefore essential for further elucidation of the pathophysiological significance of this mediator.[6-8]

In this paper we report the synthesis of some new 1,4-dihydropyridine derivatives and their activity as PAF antagonists. Additionally we studied the possible cardiovascular effects of the most potent and specific compound.

Scheme I



Chemistry

The 1,4-DHPs were prepared by using a modification of the Hantzsch synthesis reported by Fax et al.[9] as illustrated in Scheme I. The diketene–acetone adduct broke down at 100 °C to yield acetylketene (**1**); this compound is unstable and it reacted immediately with alcohol **2** to yield the β-keto ester (compound **3**).[10] Finally, the reaction of compound **3** with the corresponding 3-aminocrotonate (compound **4**) and the appropiate aldehyde (compound **5**) gave cyclized 1,4-dihydropyridine **6**.

Some alcohols of type **2** were commercially available, otherwise they were synthesized according to methods previously described.[11] The synthesis of type **3** β-keto esters has previously been reported in the literature,[12] but we obtained them, in good yield and with a high degree

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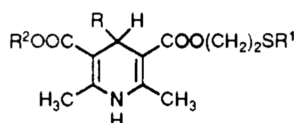
Table I. Chemical Data for 1,4-Dihydropyridines



no.	R	R^2	R^1	time, h	% yield[a]	mp, °C	cryst solv[b]	formula[c]
6A	CH_3	CH_3	CH_3CH_2	8	49	84–6	A	$C_{15}H_{23}NO_4S$
6B	CH_3	CH_3	CH_3	8	47	92–5	A	$C_{14}H_{21}NO_4S$
6C	CH_3	CH_3CH_2	CH_3	8	82	87–90	B	$C_{15}H_{23}NO_4S$
6D	CH_3	CH_3CH_2	CH_3CH_2	8	53	83–5	C	$C_{16}H_{25}NO_4S$
6E	CH_3	$CH_3O(CH_2)_2$	CH_3	12	51	58–68	A	$C_{16}H_{25}NO_5S$
6F	CH_3CH_2	CH_3CH_2	CH_3	10	65	96–8	D	$C_{16}H_{25}NO_4S$
6G	$CH_3(CH_2)_2$	CH_3CH_2	CH_3	10	56	94–6	A	$C_{17}H_{27}NO_4S$
6H	$CH_3(CH_2)_2$	CH_3CH_2	CH_3CH_2	10	62	68–70	A	$C_{18}H_{29}NO_4S$
6I	CH_3	CH_3CH_2	C_6H_5	10	49	80–2	A	$C_{20}H_{25}NO_4S$
6J	CH_3	(tetrahydrofuran-2-yl)CH_2	C_6H_5	12	65	oil		$C_{23}H_{29}NO_5S$
6K	CH_3	$(CH_3)_2CH$	C_6H_5	12	67	oil		$C_{21}H_{27}NO_4S$
6L	CH_3	CH_3	C_6H_5	8	59	74–6	C	$C_{19}H_{23}NO_4S$

[a] Refers to recrystallized product. [b] Key: A, EtOH; B, EtOH/H_2O (7:3); C, ethyl acetate. [c] Analytical data were within ±0.4% of the theoretical values.

of purity, following the procedure shown in Scheme I. Some aminocrotonates of type **4** were obtained from commercial sources, otherwise, they were prepared according to Bader et al.[13] All aldehydes (**5**) were commercially available.

Although some variations of the final reaction to obtain 1,4-dihydropyridines were examined, the best results were obtained in ethanolic medium. Reaction times between 8 and 12 h. The yields of the syntheses of 1,4-dihydropyridines were in the range of 47–82%.

Pharmacology

There are reasons to state that PAF plays a decisive role in several pathologic situations including bronchial asthma, shock, and allergy; therefore, PAF antagonists may offer potential therapeutic applications.

Our aim in the present paper is to describe the syntheses and the evaluation of a new series of 1,4-dihydropyridine derivatives showing specific PAF-antagonistic properties and lack of calcium channel blocking activity.

The compounds listed in Table I were first evaluated for PAF-antagonistic activity with binding assays employing washed rabbit platelets. The most potent compound among the 12 molecules tested was **6L**, with an IC_{50} of 33.40 nmol (K_i = 15 nmol). Nitrendipine did not show any significant effect on the binding of [^{3}H]PAF to its receptor in rabbit platelets. The IC_{50} was found to be 20 800 nmol (K_i = 7331 nmol), suggesting that PAF-antagonistic activity of this compound is a result of those nonspecific effects of the PAF receptor described for other calcium channel blockers.[14] The results of the assays on [^{3}H]nitrendipine binding to microsomes of guinea pig ileal muscle showed just the opposite: a K_d value of 4.10 nM was found for nitrendipine whereas the K_i value for compound **6L** was 568.62 nM.

At the cellular level, several compounds inhibited PAF-induced platelet aggregation and ATP release, showing a very good correlation between antiaggregatory activity and K_i for binding of [^{3}H]PAF to rabbit platelets.

In animals treated with PAF antagonist, the biological actions of PAF were blocked or reversed. Therefore, the compounds were further evaluated in anesthetized rats for their ability to reverse PAF-induced hypotension.

Table II. In Vitro PAF-Antagonistic Activity of 4-Alkyl-1,4-dihydropyridines and Standard Compounds

compd	% inhibn of platelet activation[a]: platelet aggreg	% inhibn of platelet activation[a]: release reaction	inhibn of PAF binding: K_i,[b] mol
6A	44 ± 5**	96 ± 2***	5.051×10^{-7}
6B	26 ± 7*	64 ± 18**	6.032×10^{-7}
6C	21 ± 6	68 ± 21*	1.045×10^{-6}
6D	32 ± 7**	84 ± 22*	1.001×10^{-6}
6E	0	0	4.408×10^{-6}
6F	32 ± 6**	85 ± 8***	1.012×10^{-6}
6G	14 ± 7	50 ± 15*	
6H	30 ± 10*	69 ± 11**	
6I	100 ± 0***	100 ± 0***	6.877×10^{-8}
6J	11 ± 6	49 ± 17*	2.092×10^{-6}
6K	33 ± 14	89 ± 8***	9.127×10^{-7}
6L	100 ± 0***	100 ± 0***	1.509×10^{-8}
BN-52021	99 ± 3***	100 ± 0***	6.858×10^{-7}
nitrendip	0	0	7.331×10^{-5}

[a] PAF concentration, 1.9 nmol. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. [b] PAF concentration, 0.2 nmol; experimental K_D = 0.2523 nmol.

Several compounds showed good antihypotensive activity directly related to K_i values in binding assays. The most effective compounds were **6L** and **6I**. Compounds **6G** and **6H** showed definite agonistic activity in this model.

It is clear from these data that incorporation of a thioaryl group into the chain linked to C_3 of the dihydropyridine ring (**6I–L**) leads to increased affinity for the PAF receptor and, consistently, PAF-antagonistic activity. On the other hand, it is clear, when comparing activities of compounds **6I–L**, that incorporation of a bulky substituent at C_5 of the dihydropyridine ring results in loss of activity.

Discussion

We report herein the results of the biological testing of new 1,4-DHP, listed in Table I, as PAF antagonists in a comparative study with BN-52021 (a sesquiterpene, found in *Ginko biloba*, which has been described as a specific antagonist of PAF[15]) and nitrendipine (a 1,4-dihydropyridine well-known for its calcium channel blocking activity). The compounds were tested in vitro for their

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Table III. Effects of 4-Alkyl-1,4-dihydropyridines on PAF-Induced Hypotension

compd	drop in mean arterial blood pressure, mmHg	
	3 min[a]	20 min[a]
control	71 ± 9	32 ± 7
6A	68 ± 17	32 ± 21
6B	51 ± 20*[c]	5 ± 6*[c]
6C	88 ± 19	29 ± 10
6D	64 ± 6	18 ± 4*
6E		
6F	77 ± 20	36 ± 10
6G	101 ± 18**[d]	60 ± 22*[d]
6H	106 ± 16**[d]	71 ± 15*[d]
6I	52 ± 6***	23 ± 6*
6J	63 ± 15*	21 ± 11
6K	78 ± 9	27 ± 9
6L	30 ± 8***	14 ± 9*
BN-52021	55 ± 9*	21 ± 14

[a] Time after drug or vehicle injection. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$. [b] Data represent mean ± SD of control groups in 11 experiments (five rats per group). [c] Statistical significance was calculated against values of the control group in the corresponding experiment. [d] Compounds showed clear agonistic activity.

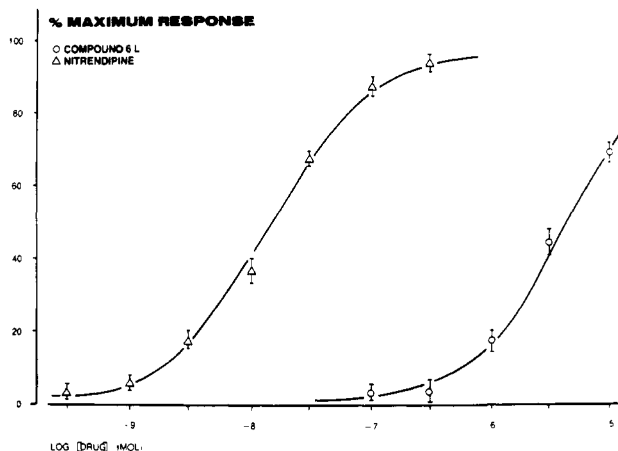

Figure 1. Inhibition of contractile response to 1.5 mM Ca^{2+} in rabbit aorta previously depolarized with 35 mM K^+. Bars represent standard error of mean (nitrendipine, $n = 10$; compound **6L**, $n = 8$).

ability to inhibit platelet aggregation and release of ATP induced by PAF-acether in washed rabbit platelets and in vivo for their ability to inhibit PAF-induced hypotension in anesthetized rats. Additionally, the equilibrium affinity constants (K_i) of compounds were determined from inhibition of specific binding of [^{3}H]PAF to rabbit platelet receptors.

The evaluation of data in Tables II and III shows that compound **6L** is the most active of the tested 1,4-DHPs, and therefore, its cardiovascular effects were assessed and compared with those of nitrendipine. Figure 1 shows the effects of the compound **6L** versus nitrendipine on vascular smooth muscle preparations of rabbit aorta. Compound **6L** showed very low inhibitory effect on contraction evoked by high K^+ concentrations (35 mmol) when compared with nitrendipine (IC_{50} = 4015.7 and 2.2 nM, respectively). Inotropic and chronotropic activities were evaluated on rabbit atria. Compound **6L** was devoid of negative inotropic actions at concentrations to 100 nM and showed only weak negative inotropic actions at 1 μM. Nitrendipine was active in both systems at 100 nM.

Figure 2 shows the hypotensive response, in anesthetized rats, to a single 300 μg/kg bolus infusion of nitrendipine as compared with that of compound **6L** (300 and 1000 μg/kg), which was inactive.

Table IV. Chemical Data for β-Keto Ester

$CH_3C(=O)CH_2COO(CH_2)_2SR$

no.	R	bp, °C/mmHg	% yield
3A	CH_3	95–8/0.5	89
3B	CH_2CH_3	79–82/0.2	92
3C	C_6H_5	104–2/0.4	88

Finally, nitrendipine and the most active compound, **6L**, were subject to binding assays including [^{3}H]PAF binding to rabbit platelets and [^{3}H]nitrendipine binding to microsomes of guinea pig ileal longitudinal muscle. These experiments allow us to validate the inactivity of compound **6L** as a calcium channel antagonist and, additionally, to show that 1,4-dihydropyridines such as nitrendipine lack activity as PAF antagonists. Results of these experiments are shown in Figure 3.

Experimental Section

Chemistry. All melting points were determined in a Büchi 510 open-capillary melting point apparatus and are uncorrected. The structures of all compounds were supported by IR and NMR spectra. IR spectra were obtained with a Perkin-Elmer 881 spectrometer and NMR spectra were recorded on a Varian T-60 spectrometer. All elemental analysis were within ±0.4%.

General Procedure for Preparation of 2-(Alkylthio)- or 2-(Phenylthio)ethyl Acetylacetate (3). Alcohols **2** were made to react with diketene–acetone adduct (**1**) to yield the corresponding β-keto ester (**3**) as is shown in Scheme I. One mol of corresponding alcohol **2** was dissolved in 400 mL of xylene. The solution was boiled under reflux and 1 mol of diketene–acetone adduct was added dropwise in order to keep the temperature below 120 °C. The mixture was stirred and heated under reflux for 1 h and the solvent was removed under reduced pressure; the residue was a liquid that was purified by vacuum distillation. Results are shown in Table IV.

General Procedure for Preparation of 2-(Alkylthio)- or 2-(Phenylthio)ethyl 5-(Alkoxycarbonyl)-4-alkyl-2,6-dimethyl-1,4-dihydropyridine-3-carboxylate (6). The reaction of β-keto esters from **3** with 3-aminocrotonates from **4** in the presence of the appropriate aldehyde RCHO (**5**) afforded the corresponding 1,4-DHP derivatives as illustrated in Scheme I. Reactions were carried out in darkness to protect the dihydropyridine ring from oxidation. A mixture of equimolar amounts of compounds **3–5** in absolute ethanol (100 mL/mol of reagent) was stirred and heated under reflux for the periods of time given in Table I. Thereafter, the reaction mixture was treated in one of the four following ways: (1) cooled to −10 °C and filtered; (2) concentrated under reduced pressure, cooled to −10 °C, and filtered; (3) solvent was evaporated under reduced pressure with a Büchi rotary evaporator and ethyl acetate added, the mixture was cooled to −10 °C, and the precipitate was dissolved in solvent and filtered; (4) when the product was an oil, it was filtered through activated charcoal/diatomaceous earth (1:1) and the solvent was evaporated under reduced pressure. Solid products obtained were recrystallized from the appropriate solvent given in Table I.

Biological Methods. In Vitro Determination of Platelet Aggregation. Citrated rabbit blood was centrifuged to prepare platelet-rich plasma (PRP), as previously described.[16] Washed rabbit platelet suspensions (WRPS) were prepared by following the slightly modified method of Vargas et al.[17] Basically, platelets were sedimented from PRP by centrifugation for 20 min at 2000g at 4 °C and washed three times with tyrode/HEPES buffer, pH 6.5, containing glucose (1 mg/mL), bovine serum albumin (3.5 mg/mL), apyrase (25 μg/mL), and PGE_1 (10 ng/mL). After that,

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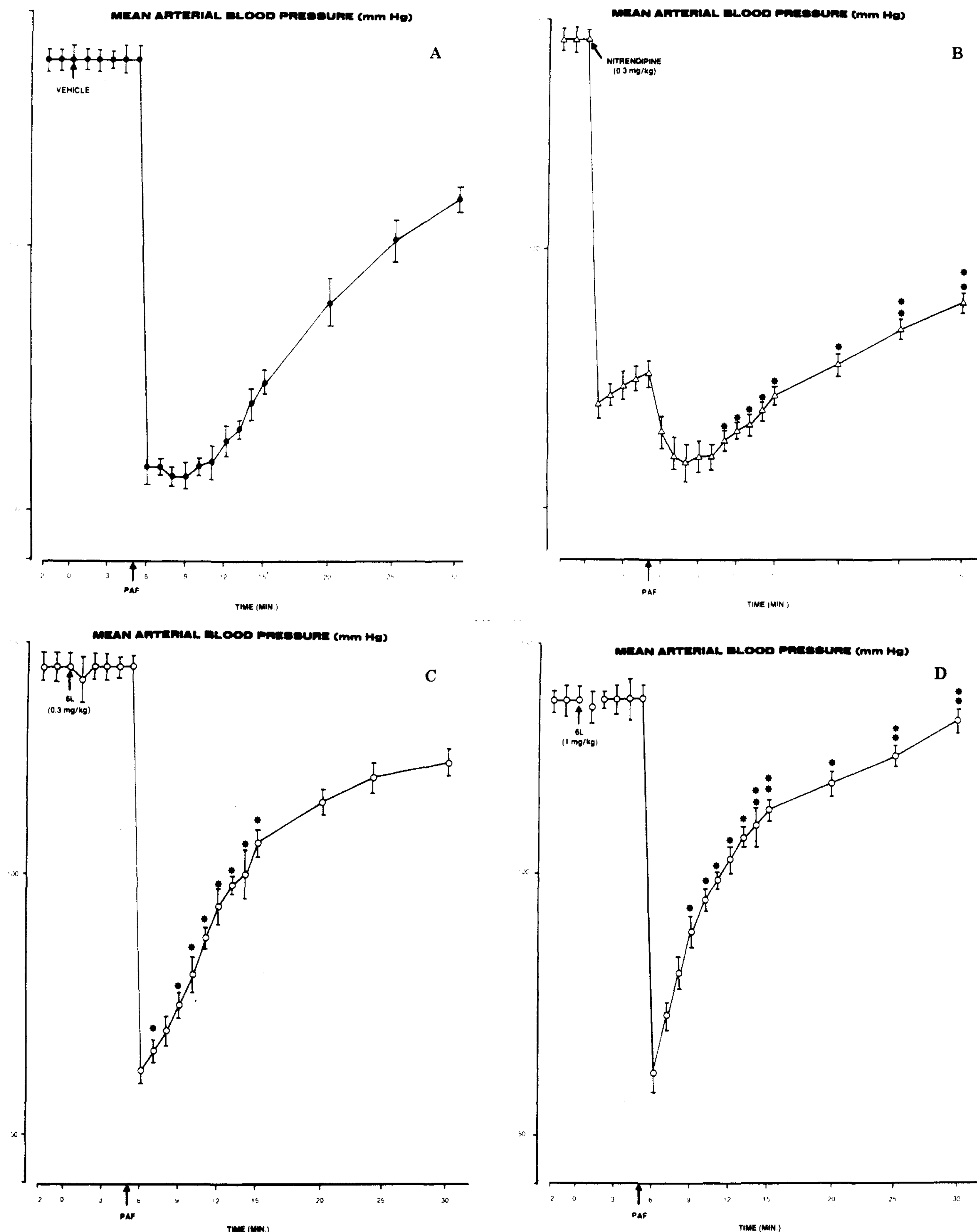

Figure 2. Reversal of PAF-induced hypotension. Compounds or vehicle were administered iv at time = 0, PAF was injected at time = 5 min. Data are the mean ± SD of five experiments: * $p < 0.05$; ** $p < 0.01$.

the platelets were sedimented by centrifuging as above and suspended in HEPES/tyrode buffer, pH 7.35, where PGE_1 and apyrase were not included. The platelet count was adjusted to 350000/μL. 450-μL samples of WRPS were preincubated, with 2 μL of solutions of compounds in dimethyl sulfoxide (DMSO)/saline (1:1) for 5 min at 37 °C before addition of PAF (1.9 nmol). DMSO was included in control samples of WRPS. Responses to PAF were quantified by measuring the maximum increase in light transmission 5 min after the addition of agonist. Final concentrations of compounds and standard drugs were 10 μmol.

Release of ATP. ATP release from platelets, as a response to activation by agonists, was measured simultaneously with platelet aggregation in a Lumi-aggregometer (Chrono-Log Co.,

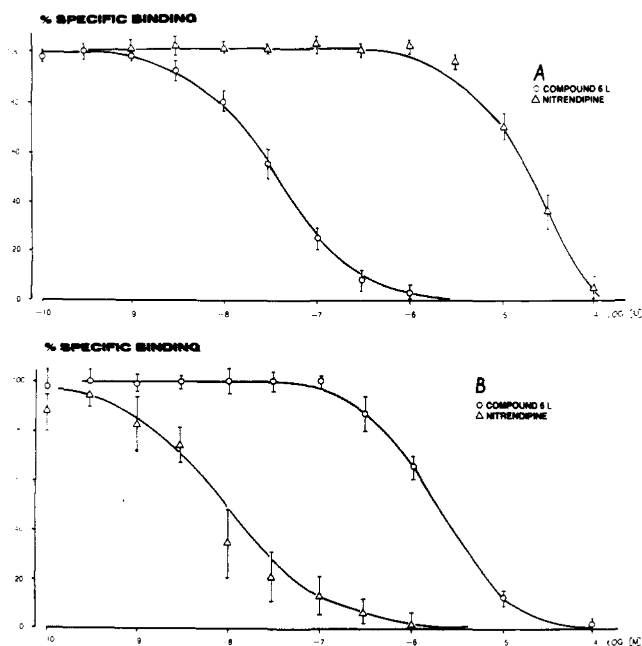

Figure 3. (A) Displacement of [^{3}H]PAF specifically bound to washed rabbit platelets by unlabeled nitrendipine and compound **6L**. (B) Inhibition of specific [^{3}H]nitrendipine binding to guinea pig ileal longitudinal muscle microsomal fractions. Bars represent standard deviation of mean (n = 6 experiments in triplicate for A; n = 3 experiments in triplicate for B).

Haventon, PA) by using a luciferin/luciferase reagent.[18]

Effects of Compounds on [^{3}H]PAF Binding to Washed Rabbit Platelets. The experiments were performed according to the method of Valone[19] with slight modifications. The washed platelets were resuspended at a concentration of 2.0 × $10^5/\mu$L in a binding buffer (Ca^{2+}-free tyrode solution, pH 7.25, containing 0.2% (w/v) bovine serum albumin and 5 mM glucose). No stimulation or inhibition of radiolabeled PAF was found with the addition of 2 mM $CaCl_2$; therefore Ca^{2+} was not added in the assay even though it will potentiate the PAF-induced platelet activation.

Platelet suspensions (500 μL) were added to siliconized test tubes and incubated with [^{3}H]PAF (0.2 nmol) and antagonist (10^{-5}–10^{-10} mol) for 30 min at room temperature. The binding reaction was stopped by cooling the samples to 0 °C. Platelets were isolated by vacuum filtration on glass filters (Whatman GF/C). The filters were presoaked with the washing solution. Each tube and filter was rapidly washed three times with 3 mL of ice-cold washing solution. The filtration procedure was complete within 15 s. The corresponding values for radioactivity linked to the filters were measured by simultaneous processing of samples without platelets. They were always under 0.6% of total radioactivity bound to platelets. No displaceable binding was calculated as the remaining binding in the presence of 2 μmol of nonlabeled PAF. Specific binding was 65–75% of the total binding for every experiment.

Effects of Nitrendipine vs 6L on [^{3}H]Nitrendipine Binding to Microsomes of Guinea Pig Ileal Longitudinal Muscle. The experiments were performed according to the method of Bolger et al.[20] Guinea pig ileal longitudinal muscle was isolated, and microsomes were prepared and resuspended in cold 50 mM Tris buffer, pH 7.4, containing 0.1 mM methyl sulfoxide, at a concentration of 10–20 μg/mL. Protein concentration was determined by the method of Lowry[21] with bovine serum albumin as standard.

Protein suspension (1 mL) containing 10–20 μg of membrane protein was incubated with [^{3}H]nitrendipine (0.8 nmol) and drugs (10^{-4}–10^{-10} mol) for 60 min at room temperature. After incubation, samples were filtered under vacuum through Wathman GF/B filters and the filters were washed twice under vacuum with 5-mL portions of cold Tris buffer. The filters were treated and counted as above.

Nonspecific binding was measured in the presence of 1 μmol of unlabeled nitrendipine. Nonspecific binding was subtracted from total binding to tissues and filters to obtain specific binding. The latter was 50–70% of the total binding for every experiment.

Studies on Anesthetized Rats. The methodology for PAF-induced hypotension has been described previously.[22] Male Sprague–Dawley rats, 225 ± 2 g of body weight, anesthetized with sodium pentobarbital (50 mg/kg ip) were used. The mean arterial blood pressure was recorded on a polygraph via a pressure transducer (Statham P23 Db) from a polyethylene cannula inserted into the right carotid artery. PAF and test compounds were given via a polyethylene catheter inserted into a femoral vein in a volume of 0.2 mL/kg of polyethylene glycol 400/silane (1:9). In the first series of experiments, compounds (5 mg/kg) were given intravenously when the blood pressure was maximally reduced, 3 min after PAF (0.66 μg/kg) had been injected. The changes in blood pressure were recorded for 30 min after PAF administration.

In the second series of experiments, the most active compound, **6L**, or nitrendipine were given at 1 and 0.3 mg/kg doses 5 min before PAF was injected. Changes in mean arterial blood pressure were recorded for 30 min and compared with those of rats receiving vehicle alone (control group). This design allowed us to establish in the same animal the anti-PAF effect of compound **6L** and the lack of any hypotensive activity as compared with nitrendipine, a calcium channel blocker with well-known hypotensive effects. Results are shown in Figure 3.

Vascular Effects. The experimental protocol is similar to another previously described.[23,24] Male New Zealand rabbits weighing 1.5–2.5 kg were sacrificed and the thoracic aorta was removed and placed in Krebs–bicarbonate buffer. Excess of fat and tissue were removed, and the aorta was cut in helicoidal strips.[25] Strips were mounted in organ baths under a 2.5-g preload, which was applied in all experiments. Bath temperature was kept at 37 °C and Krebs–bicarbonate was bubbled with 95% O_2 + 5% CO_2 (final pH 7.4). The segments of aorta were kept in Krebs solution without calcium during 1 h for equilibration. The aorta was washed every 20 min to avoid interference of metabolites.[26] After this time, a depolarization with 35 mmol of K^+ was induced, and 10 min later 1.5 mmol of Ca^{2+} was added to evoke a contraction. This process was repeated until a reproducible response was obtained. Then, strips were exposed to increasing concentrations of compound **6L** or nitrendipine. Relaxant responses were recorded and normalized with respect to initial recorded tensions. IC_{50} values were determined from concentration-response curves by the method of Finney.[27]

Inotropic and Chronotropic Effects. Male New Zealand rabbits weighing 2–2.5 kg were sacrificed by cervical dislocation. After thoracotomy the hearts were immediately removed, and the atria were separated from the ventricles and mounted in organ baths (40 mL) containing bicarbonated Krebs-Henseleit buffer gassed with 95% O_2 and 5% CO_2. The bath was kept at 32 °C. Inotropic responses were recorded from electrically stimulated left atria by a GRASS S-9 stimulator (1 Hz, 10 ms and double threshold voltage). Right atria were used in studies on the chronotropic activity. Left and right atria were allowed to equilibrate for 1–1.5 h before starting the experiment, and then compounds were added to the bath in accumulative doses

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(10^{-10}–10^{-6} mol). Stock solutions of compound **6L** and nitrendipine (0.01 mol) were prepared in DMSO and then diluted in Krebs-Henseleit buffer. Final concentration of DMSO in organ bath was always <0.1%. The isometric contraction was measured continuously by using a LETICA force displacement transducer and recorded on a GRAPHIC 1002 (LLOYD Instrument) recorder. The values of rate and contractile force were expressed as percent of variation with respect to control values.

Statistical Methods. Paired Student's *t* test was used to show any significant difference.

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Structure–Activity Relationship of Antiestrogens. Studies on 2,3-Diaryl-1-benzopyrans[†]

Ashraf Saeed,[§] Arun P. Sharma,[∥] Neelam Durani,[‡] Raka Jain,[⊥] Susheel Durani,*[,‡] and Randhir S. Kapil[#]

Medicinal Chemistry Division, Central Drug Research Institute, Lucknow - 226 001, India. *Received April 5, 1990*

A series of 2,3-diaryl-1-benzopyran analogues substituted at position 4 of 2-phenyl with a hydroxy or pyrrolidinoethoxy residue were synthesized as models for (*E*)-triarylpropenones constrained in the s-trans conformation. The prototypes, belonging to five chemical series, were evaluated for their estrogen receptor affinity and for estrogen agonist–antagonist activities. The 4*H*-1-benzopyran-4-one, the 2,3-dihydro-4*H*-1-benzopyran-4-one, the 4*H*-1-benzopyran, and the 2,3-dihydro-1-benzopyran derivatives were found to be inactive or only marginally activate as receptor ligands or estrogen agonists–antagonists. In the 2*H*-1-benzopyran category the parent phenol was also inactive whereas the basic ethers **16** and **26** were modest receptor ligands while being quite active as antiestrogens. In a comparative study the benzopyran **16** was found to be more effective antiestrogen than tamoxifen while being as effective as LY-117018. The benzopyrans have thus emerged as a new class of potent antiestrogens.

Introduction

The triarylethylene (TAE) antiestrogens represented by tamoxifen (Chart I) are well known for being associated with marked agonist activity.[1-6] The (*Z*)-triarylpropenones (*Z*-TAPs), such as trioxifen, LY-117018, and LY-139481 (Chart I), though better antiestrogens than TAEs, are also associated with some residual agonist character.[7-10] Although Wakeling and Bowler reported recently the development of 7α-substituted estradiols as "pure" antiestrogens,[11,12] the link between molecular structure of antiestrogens and their residual agonist character has remained an obscure one. Our efforts in this area have thus focused on structure–activity relationship (SAR) among TAEs and TAPs so as to better understand this link.

In a recent study on SAR of *Z*-TAPs, using acyclic propenones as models, we discovered that *E*-TAPs were also associated with significant receptor affinity with some of the compounds more effective ligands than the *Z* isomers.[13] A follow-up study based on conformationally constrained models suggested that *E*-TAPs could interact with the receptor through their s-cis conformation but that such a binding mode was unlikely to account for their action as antagonists.[14]

Upon careful reappraisal the previously published data on relative binding affinity (RBA) of *E*-TAPs[14] suggested the alternate possibility that the prototype may interact with the receptor through s-trans conformation such that its C-1 and C-2 aryls simulate the Ph and Ph′ residues of

Chart I

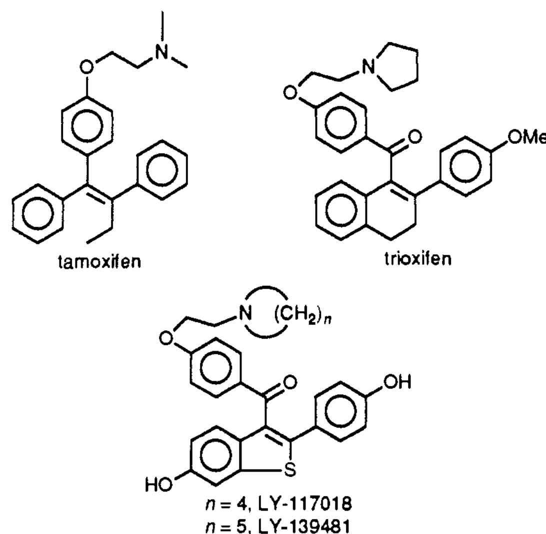
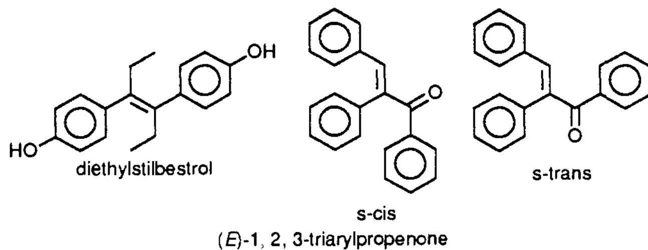


Chart II



[†] C.D.R.I. Communication Number 4341.

[‡] Present address and address for correspondence: Biosciences and Engineering Group, Chemistry Department, Indian Institute of Technology, Powai, Bombay - 400 076, India.

[§] Present address: Department of Chemistry, University of Sussex, Brighton, UK.

[∥] Present address: Department of Chemistry, Florida A&M University, Tallahassee, FL.

[⊥] Present address: Department of Psychiatry, All India Institute of Medical Sciences, New Delhi, India.

[#] Regional Research Laboratory, Jammu, India.

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