Design and Synthesis of Piperidine-3-carboxamides as Human Platelet Aggregation Inhibitors

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A detailed structure–activity analysis was carried out using eight 1-alkyl(aralkyl)nipecotamides (type **5**), 33 bis-nipecotamidoalkanes and aralkanes (type **6**), and 7 *N,N'*-bis(nipecotoyl)-piperazines (type **7**) as inhibitors of human platelet aggregation. Steric factors played an important role in determining the activity of type **5** compounds possessing an appropriate degree of hydrophobic character. Types **6** and **7** compounds were more potent than the corresponding type **5** molecules. Hydrophobic character appeared to influence the activity of type **6** compounds. A 3-substituent on the piperidine ring was necessary for antiplatelet activity; the substituent should be preferably an amide with its C attached directly to the ring. 3,5-Disubstitution and 2-substitution led to a decline in activity. Optimal activity was attained when the two nipecotoyl ring N atoms were connected by an aralkyl group, and separated by ~7 Å. It is suggested that van der Waals forces and π interactions may govern the inhibitor–platelet interaction. The most potent type **6** inhibitor was α,α'-bis[3-(*N*-ethyl-*N*-butylcarbamoyl)piperidino]-*p*-xylene (**6i**). The most potent type **5** compound was 1-decyl-3-(*N,N*-diethylcarbamoyl)piperidine (**5a**). Any substitution on the piperazine ring of type **7** compounds led to a decline in activity, the most active analog being *N,N'*-bis(1-decylnipecotoyl)piperazine (**7a**). It is suggested that nipecotamides interact with anionic platelet sites located 7 Å from each other and connected by a hydrophobic well.

Platelets play a pivotal role in the pathogenesis of thrombosis and atherosclerosis.[1] They are activated upon exposure to collagen of the damaged endothelium, or to a fractured atherosclerotic plaque,[1b] or upon contact with a biomaterial,[2] resulting in the release of agonists such as adenosine diphosphate (ADP) and thromboxane A_2 (TxA_2). These agonists induce further activation of surrounding platelets leading to aggregation, and eventually to the formation of a stable thrombus. Agents which inhibit the aggregation of platelets have been employed in the prevention and therapy of thrombotic disease.[3] During our investigations in search of antiplatelet agents for use in the prevention of biomaterial-induced thrombosis, we discovered a piperidine-3-carboxamide (nipecotamide) **6c**, which inhibited human platelet aggregation (induced by ADP,[4] collagen,[5] thrombin,[6] epinephrine,[7] and the stable TxA_2-mimetic U46619) *in vitro*,[8] in dogs *ex vivo*,[9] and blocked dacron-induced thrombus formation in baboons *in vivo*.[10] In protecting mice from thromboembolic death caused by the intravenous injection of collagen+ epinephrine, **6c** and its analog **6l** were quite potent, with ED_{50} values of 104 and 20 mg/kg respectively; further, their *(meso)-R,S*-diastereomers were more active than the racemates, the ED_{50} values being 33.5 and 11 mg/kg for **6c** and **6l**, respectively.[11] In inhibiting collagen-induced human platelet aggregation *in vitro*, the IC_{50} values of these two diastereomers were 10.7 and 1.0 μM, respectively.[5d] On the basis of experiments currently in progress, (*R,S*)-**6c** appears to be effective in blocking platelet aggregation *ex vivo* in dogs administered doses lower than 12 mg/kg. These molecules offered attractive leads since they possess several sites with potential for structural manipulation. Systematic modification of this structure yielded superior antiplatelet compounds **6d**, **6f**, **6i**, and **6o**. This report deals with a discussion of the structure–activity relationships among three types (**5**, **6**, and **7**) of analogs of **6c** and other nipecotamides (Scheme 1).

Chemistry

Three structural types of nipecotamides were chosen for synthesis: 1-Alkyl(aralkyl)nipecotamides (type **5**) having one piperidine (tertiary) N and a 3-amide group. The N was designed to carry a hydrophobic substituent. Bisnipecotamidoalkanes and aralkanes (type **6**) carrying 2 tertiary N atoms separated by 7–8 Å were designed such that the flexibility variance (due to buckling of aliphatic hydrocarbon chains) in the interatomic distance between the two tertiary nitrogen atoms varied among congeners. It was postulated that these two nitrogen atoms interact with complementary sites in the platelet subsequent to protonation.[12,13] The third type (**7**) was *N,N'*-bis(1-alkyl)piperazine where the interatomic distance between the two tertiary N atoms is locked in. The general synthetic routes for these compound types are shown in Scheme 1. Condensation of the appropriate amine with nicotinoyl chloride gave the corresponding nicotinamide (**1**), which upon the addition of a 1-bromo(or 1-iodo)alkane, or α-bromoaralkane afforded the quaternary intermediate **2**, the PtO_2/H_2 reduction of which gave **5a**–**h**. In the synthesis of **5g**, *N*-(2-bromoethyl)phthalimide was condensed with **1** to give **2g** (R'' = *N*-phthalimidoethyl). Reaction of **1** with α,α'-dibromoaralkane yielded the intermediate **3**, which upon catalytic reduction afforded compounds **6a**–**ah**. Condensation of nicotinoyl chloride (2 molar equiv) with (1 molar equiv of) piperazine followed by *N*-alkylation of **4** and catalytic reduction of the resultant quaternary intermediate afforded **7a**–**g**. In obtaining

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Scheme 1

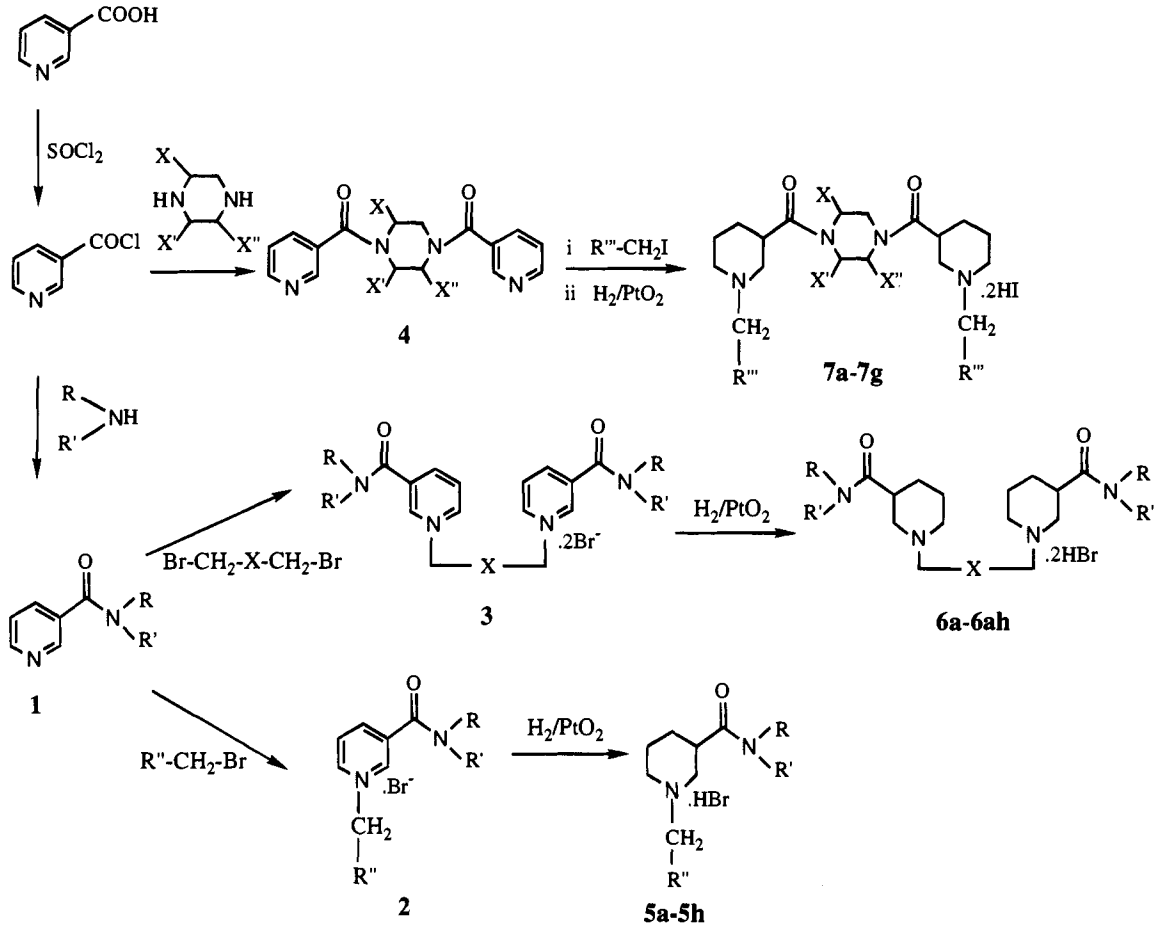


Table 1. Analytical and Physical Data of 1-Alkyl(or aralkyl)-*N,N*-diethylnipecotamides (Type **5** Compounds)

compd no.	X	recryst solvent	mp, °C	formula[a]	MS (MH^+)
5a	C_9H_{19}		157.5–158.0[b]		
5b	*p*-Cl-C_6H_5	EtOH	251.5–252.8	$C_{17}H_{26}N_2OClBr$	309
5c[c]	C_9H_{19}	EtOAc:*n*-C_6H_{13} (1:1)	122.6–123.5	$C_{20}H_{41}N_2SCl$	341
5d[d]	*m*-ClC_6H_5	CH_3CN:EtOAc (20:1)	220.1–221.0	$C_{17}H_{26}N_2OClBr$	309
5e	*p*-*t*-BuC_6H_5	CH_3CN:EtOAc (1:8)	132.8–134.1	$C_{21}H_{35}N_2OBr$	331
5f	*o*-ClC_6H_5	EtOAc:CH_3COCH_3 (9:1)	146.5–147.7	$C_{17}H_{26}N_2OClBr$	309
5g	CH_2CH_2N	EtOH	189.4–189.8	$C_{20}H_{34}N_3O_3Br$	364
5h	C_6H_5	CH_3CN	213.0–214.0	$C_{17}H_{27}N_2OBr$	

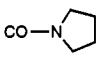
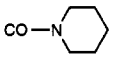
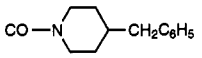
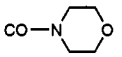
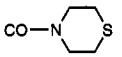
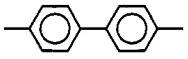
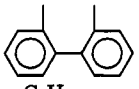
[a] The elemental analyses were within ±0.4% of the theoretical values for C, H, and N. Halogens and S were analyzed when present. [b] From ref 12. [c] C_3 Substituent is *N,N*-diethylthioamide [(C=S)NEt_2]: IR (KBr) 1085 cm^{-1} (ν C=S); ^1H NMR (CD_3OD) δ ppm 2.91–4.12 (m, 11H, piperidine $CH_2N[CH_2R]CH_2CHCON[CH_2CH_3]_2$), 1.78–2.02 (m, 4H, piperidine $NCH_2CH_2CH_2CHCO$), 1.19–1.37 (m, 22H, $NCH_2[CH_2]_8CH_3$, $N[CH_2CH_3]_2$), 0.89 (t, 3H, J = 7 Hz, $N[CH_2]_9CH_3$). [d] (^1H) NMR ($CDCl_3$) δ ppm 7.35 (s, 1H, Ar C_2H), 7.16–7.23 (m, 3H, Ar*H*), 3.48 (s, 2H, NCH_2Ar), 3.26–3.41 (m, 5H, piperidine CH_2NCH_2CHCO), 2.68–2.87 (m, 4H, $N[CH_2CH_3]$), 1.53–1.79 (m, 4H, piperidine $NCH_2CH_2CH_2CHCON$).

7f, homopiperazine was used in place of piperazine. In the preparation of **7g**, *N*-(2-bromoethyl)phthalimide was treated with **4g** (X, X', and X'' = H). In the synthesis of **6ah**, nicotinoyl choride was treated with 3-aminopyridine to give *N*-(3-pyridyl)nicotinamide, which was condensed with 1-bromodecane followed by catalytic hydrogenation (PtO_2/H_2). The preparation of 1-*tert*-butyl-3-(*N,N*-diethylcarbamoyl)piperidine hydrobromide (**5e**) and *N,N'*-Bis (1-decylnipecotoyl) 2-methylpiperazine (**7b**) are described (in the Experimental section) as representative examples of types **5** and **7** compounds. The synthesis of type **6** compounds followed the same general outline and was described earlier.[13] The synthetic compounds were characterized by elemental analyses, and where indicated, by NMR, IR, and mass spectrometry. The analytical and physical characteristics of the new compounds are summarized in Tables 1 for type **5**, 2 for type **6** and 3 for type **7**. The NMR data of representative compounds are given in Tables 1 and 2 and, where appropriate, in the Experimental Section.

Biological Results and Discussion

Nipecotamides inhibited the primary phase of aggregation, believed to be related to receptor-associated initial events, as well as the secondary, irreversible phase of aggregation linked to the release reaction (Figure 1). Typical aggregation tracings from experiments with two different compounds are also shown in Figure 1. Figure 1A shows 58.3% inhibition of primary as well as secondary phases by 200 μM of **6ab** (IC_{50} 134.2 μM ± 28.4 SE). Similarly, Figure 1B shows 51% inhibition of total aggregation by 12.5 μM of **6i** (IC_{50} 18.5 μM ± 6.2 SE). At higher concentration of nipeco-

Table 2. Analytical and Physical Properties of Bis(nipecotamido)alkanes and -aralkanes (Type **6** Compounds)

compd no.	X	R	recryst solvent	mp, °C	formula[a]	MS (MH^+)
6a	*p*-C_6H_4	H	MeOH	316.0 dec	$C_{18}H_{30}N_2Br_2$	
6b	*p*-C_6H_4	CH_3	MeOH	325.0–325.7	$C_{20}H_{34}N_2Br_2$	
6c[b]	*p*-C_6H_4	$CONEt_2$	EtOH	278.0–279.0	$C_{28}H_{48}N_4O_2Br_2$	
6d	*p*-C_6H_4	CON(Me)*n*-Pr	EtOH:EtOAc (1:1)	233.5–234.5	$C_{28}H_{48}N_4O_2Br_2$	
6e	*p*-C_6H_4	CON(Me)*i*-Pr	EtOH:EtOAc (3:4)	258.8–260.3	$C_{28}H_{48}N_4O_2Br_2$	
6f[b]	*p*-C_6H_4	CON(Me)*n*-Bu	n-PrOH	257.0–258.0	$C_{30}H_{56}N_4O_2Br_2$	
6g	*p*-C_6H_4	CON(*n*-Pr)$_2$	C_2H_5OH	271.0–271.8	$C_{32}H_{56}N_4O_2Br_2$	
6h	*p*-C_6H_4	CON(*i*-Pr)$_2$	EtOH	283.5–284.0	$C_{32}H_{54}N_4O_2Br_2$	
6i	*p*-C_6H_4	CON(Et)*n*-Bu	EtOH	248.2–249.2	$C_{32}H_{56}N_4O_2Br_2$	527.4
6j	*p*-C_6H_4	CON(Me)*n*-Hex	EtOH:EtOAc (1:1)	250.0–252.0	$C_{34}H_{60}N_4O_2Br_2$	
6k[b]	*p*-C_6H_4	CON(H)Bz	*n*-PrOH	170.0–190.2		
6l[b]	*p*-C_6H_4	CON(CH_3)Bz	EtOH:EtOAc (1:1)	251.5–252.3		
6m[b]	*p*-C_6H_4	CON(Et)Bz	EtOH	279.2–280.5		
6n[b]	*p*-C_6H_4	CON(Bz)$_2$	EtOH	259.0–260.0		
6o	*p*-C_6H_4		*i*-PrOH:MeOH (1:0.66)	282.5–283.5	$C_{28}H_{44}N_4O_2Br_2$	
6p	*p*-C_6H_4		EtOH	285.0–286.0	$C_{30}H_{48}N_4O_2Br_2$	
6q	*p*-C_6H_4		*i*-Pr:EtOH (10:1)	248.0–249.0	$C_{42}H_{60}N_4O_2Br_2$	
6r	*p*-C_6H_4		MeOH:EtOAc (10:1)	314.0–315.0	$C_{28}H_{44}N_4O_4Br_2$	499
6s	*p*-C_6H_4		MeOH	277.0–278.0	$C_{28}H_{44}N_4O_2S_2Br_2$	531.4
6t	$(CH_2)_6$	CON(Et)$_2$	EtOH:EtOAc (3:4)	226.6–227.8	$C_{28}H_{56}N_4O_2Br_2$	480
6u[c]	$(CH_2)_8$	CON(Et)$_2$				
6v	$(CH_2)_{10}$	CON(Et)$_2$	EtOH:EtOAc (5:8)	228.3–229.7	$C_{32}H_{64}N_2O_2Br_2$	536
6w[d]	*p*-C_6H_4	3,5-[CON(Et)$_2$]$_2$	EtOH	310 (chars)	$C_{38}H_{64}N_6O_4Br_2 \cdot 2H_2O$	669.5
6x	*p*-C_6H_4	CH(OH)CH_3	MeOH:EtOAc (1:1)	268.3–269.8	$C_{22}H_{38}N_2O_2Br_2$	361
6y	cyclohexyl	CON(Et)$_2$	acetone:MeOH (1:1)	295.0–296.0	$C_{28}H_{56}N_4O_2Br_2$	
6z		CON(Et)$_2$	CH_3CN:EtOAc (1:3)	210.3–211.8	$C_{34}H_{52}N_4O_2Br_2$	547
6aa		CON(Et)$_2$	CH_3CN:EtOAc (5:7)	209.8–210.9	$C_{34}H_{52}N_4O_2Br_2$	547
6ab	*p*-C_6H_4	CONH(3,4,5-tri-OMe)Bz	EtOH	243.0–244.0	$C_{40}H_{56}N_4O_8Br_2$	
6ac[e]	*p*-C_6H_4	CON(Et)$_2$	*n*-PrOH	236.5–237.5	$C_{30}H_{52}N_4O_2Br_2$	
6ad	*p*-C_6H_4	$CH_2NHCOCH_3$	MeOH:EtOAc (2:1)	279.1–279.8	$C_{24}H_{40}N_4O_2Br_2$	415
6ae[f]	*p*-C_6H_4	COOEt	EtOH	228.4–229.5	$C_{24}H_{36}N_2O_4Br_2$	417
6af[g]	*p*-C_6H_4	C(=)SN(Et)$_2$	MeOH	306.4–307.1	$C_{28}H_{48}N_4S_2Cl_2$	503
6ag[h]	*p*-C_6H_4	COOH	H_2O	250.6–251.9	$C_{20}H_{30}N_2O_4Cl_2$	361
6ah[i]			CH_3COCH_3:MeOH (1:1)	270.0–271.0	$C_{31}H_{63}N_3OI_2$	

[a] See footnote *a*, Table 1. [b] See ref 14. [c] See ref 13. [d] α,α′-Bis[3,5-bis(*N*,*N*-diethylcarbamoyl)piperidino]-*p*-xylene dihydrobromide. [e] α,α′-Bis[3-(*N*,*N*-diethylcarbamoyl)-2-methylpiperidino]-*p*-xylene dihydrobromide. [f] ^1H NMR (DMSO-d_6) δ ppm 7.67 (s, 4H, Ar*H*), 4.41 (s, 4H, NCH_2PhCH_2N), 4.07 (q, 4H, *J* = 7 Hz, COOCH_2CH$_3$), 2.86–3.53 (m, 10H, piperidine CH_2NCH_2C*H*CHCO), 1.44–2.04 (m, 8H, piperidine NCH$_2$CH_2CH_2CHCO), 1.15 (t, 6H, *J* = 7 Hz, COOCH$_2$CH_3). [g] ^1H NMR (CD_3OD) δ ppm 7.70 (s, 4H, Ar*H*), 4.87 (s, 4H, NCH_2ArCH_2N), 4.01–4.68 (m, 4H, piperidine NCH_2CH), 3.32–3.88 (m, 12H, N[CH_2CH$_3$]$_2$, piperidine CH_2NCH$_2$CH), 2.85–3.20 (m, 2H, C*H*C=SN), 1.74–2.10 (m, 8H, piperidine NCH$_2$CH_2CH_2CH), 1.02–1.37 (m, 12H, N[CH$_2$CH_3]$_2$). [h] ^1H NMR (D_2O) δ ppm 7.45 (s, 4H, Ar*H*), 4.27 (s, 4H, NCH_2ArCH_2N), 3.34–3.37 (m, 4H, piperidine NCH_2CH), 2.61–3.00 (m, 6H, piperidine CH$_2$CH_2NCH$_2$C*H*), 1.34–2.03 (m, 8H, piperidine CH$_2$NCH_2CH_2CH$_2$CH$_2$CO). [i] 3-(1-(Decylnipecotamido)-1-decylpiperidine.

Table 3. Analytical and Physical Data on *N*,*N*′-Bis(1-alkylnipecotoyl)piperazines (Type **7** Compounds)

compd no.	X	X′	X″	R	recryst solvent	mp, °C	formula[a]	MS (MH^+)
7a[b]	H	H	H	$C_{10}H_{21}$				
7b	Me	H	H	$C_{10}H_{21}$	EtOH	214.0–215.0	$C_{37}H_{72}N_4O_2I_2$	
7c	Me	H	Me	$C_{10}H_{21}$	EtOH:H_2O (4:1)	256.0–257.0	$C_{38}H_{76}N_4O_2I_2$	
7d	Me	Me	H	$C_{10}H_{21}$	EtOH:H_2O (6:1)	254.5–255.5	$C_{38}H_{76}N_4O_2I$	
7e[c]				$C_{10}H_{21}$	EtOH	258.0–259.0	$C_{42}H_{80}N_4O_2I_2$	
7f[d]				C_5H_{11}	CH_3COCH_3	244.4–245.5	$C_{27}H_{52}N_4O_2Br_2$	
7g[e]					MeOH	300.4–303.1	$C_{36}H_{56}N_6O_6Br_2$	667.4

[a] See footnote *a*, Table 1. [b] From ref 6. [c] 1,1′-Bis(*N*-decylnipecotamido)-4,4′-bipiperidine. [d] Homopiperazine in place of piperazine moiety. [e] *N*,*N*′-Bis[1-(2-phthalimidoethyl)nipecotoyl]piperazine dihydrobromide.

tamides, both phases were totally eliminated.[5d] The platelet aggregation–inhibitory activities of types **5**, **6**, and **7** nipecotamides are presented in Tables 4, 5, and 6, respectively. The IC_{50} (μM) values represent the mean ± SE of four to six individual determinations. Only one determination was made in many cases where the IC_{50} value was >500 μM. The activities of some compounds had to be determined using ethanolic (95%) solutions. It has been reported that total ethanol concentration up to 0.19% had no effect on platelet aggregation.[14] In general, the bisnipecotoylalkanes and -aralkanes were more potent than their corresponding mononipecotoyl congeners, in agreement with earlier findings.[12] Thus, **6u**, a type **6** compound, was more

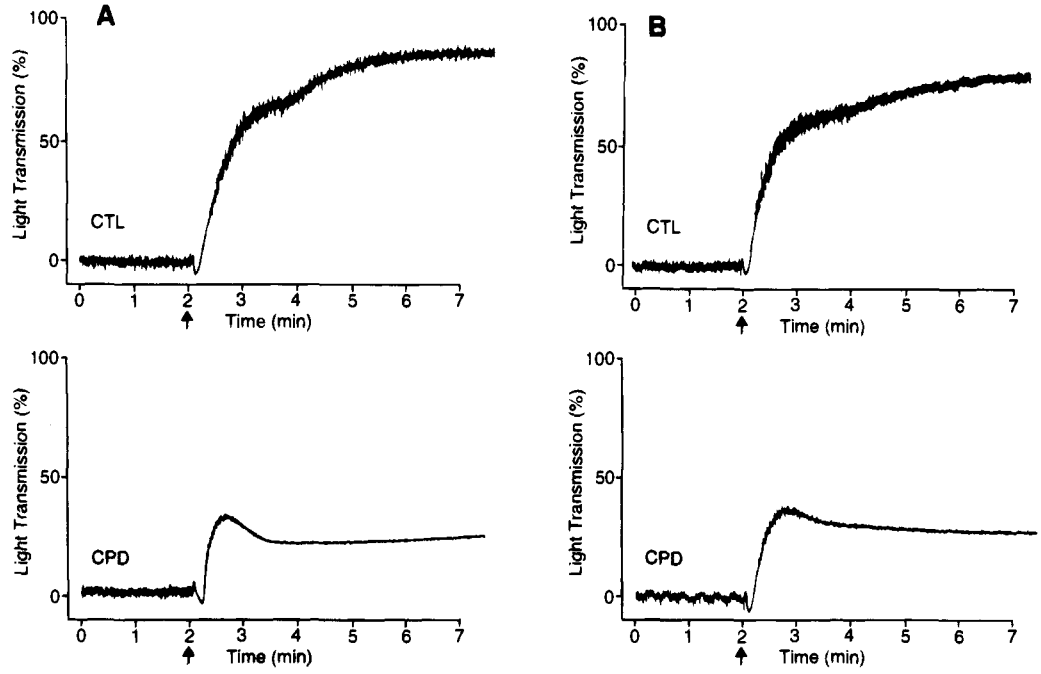


Figure 1. Representative tracings of the inhibition of ADP-induced aggregation by (A) 200 μM 6ab and (B) 12.5 μM **6i**. ADP was added at the time indicated by the arrow.

Table 4. Human Platelet Aggregation Inhibitory Activities and Partition Coefficients of Type **5** Compounds

compd no.	log P^a	IC_{50} (μM ± SE)	n
5a	5.76	241.9 ± 27.6	4
5b	3.49	216.6	1
5c	6.53	358.4 ± 97.5	4
5d	3.49	1652.8 ± 102.4	4
5e	4.83	inactive[b]	
5f	3.49	2805.5 ± 144.5	2
5g	2.43	3250.0	1
5h	2.75	7399	1

[a] Octanol/water partition coefficients. [b] 2% inhition at 200 μM.

active (IC_{50} 207.0 μM) than the corresponding type **5** compound, **5a** (IC_{50} 241.9 μM).

Type 6 Compounds. A detailed structure–activity relationship analysis was carried out using 33 type **6** bisnipecotoylalkanes and -aralkanes. The antiplatelet potency of nipecotamides appeared to be determined to a large extent by their lipophilicity, nature of the piperidine ring's 3-substituent, and the distance between the two piperidine N atoms (in bisnipecotoyl derivatives).

Hydrophobicity. The activity of type **6** nipecotamides appeared to be governed to some extent by their hydrophobicity. It may be seen from Table 5 that the most active type **6** nipecotamides (IC_{50}, μM/log P) were **6i** (18.4/5.55), **6f** (22.1/4.52), **6l** (27.3/4.72), **6p** (28.9/3.79), **6o** (32.7/2.75), **6d** (34.6/3.48), **6m** (37.7/5.76), **6g** (39.3/5.55), and **6c** (44.5/3.48). The dependency of activity upon octanol/water partition coefficients was examined using 13 type **6** nipecotamides. Only *N,N*-disubstituted compounds were included in the calculations. This relationship appears to be applicable only when the amide N is disubstituted. Compounds with the amide N carrying a H atom were relatively weak inhibitors of aggregation. Intra- and intermolecular H-bonding is possible in such structures, and this renders the amide O less amenable to interactions with platelet receptor/membrane sites.[6,9,15] Also, those with the 3-amide N as part of a heterocyclic ring were not included.

Table 5. Human Platelet Aggregation Inhibitory Activities and Partition Coeffficients of Type **6** Compounds

compd no.	log P^a	IC_{50} (μM ± SE)	log $1/C^b$	n
6a	3.57	8463.0	−0.928	1
6b	4.60	>500	0.616	2
6c[c]	3.48	44.5 ± 12.7	1.352	6
6d	3.48	34.6 ± 4.8	1.461	4
6e	3.48	144.3 ± 9.0	0.268	4
6f[c]	4.52	22.1 ± 5.5	1.656	6
6g	5.55	39.3 ± 7.1	1.406	4
6h	5.55	58.8 ± 10.1	1.231	4
6i	5.55	18.4 ± 6.2	1.734	5
6j	6.59	48.8 ± 9.1	1.312	4
6k[c]	4.18	53.6 ± 5.3	1.271	6
6l[c]	4.72	27.3 ± 3.2	1.56	7
6m[c]	5.76	37.7 ± 4.6	1.424	7
6n[c]	8.03	302.6 ± 60.8	0.519	5
6o	2.75	32.7 ± 7.3	1.486	4
6p	3.79	28.9 ± 9.0	1.540	5
6q	8.14	277.1 ± 20.1	0.557	5
6r	0.74	1653.2 ± 1150.7	−0.218	6
6s	2.89	310.7 ± 43.4	0.507	4
6t	4.89	213.9 ± 99.6	0.670	4
6u[d]	5.92	207.0 ± 18.3	0.684	5
6v	6.96	85.3 ± 8.8	1.069	4
6w	3.39	inactive		1
6x	2.30	589.4	0.230	1
6y	4.57	>1000		1
6z	5.43	478.5 ± 32.4	0.320	4
6aa	5.43	353.5 ± 62.7	0.499	4
6ab	4.06	134.2 ± 28.4	0.872	6
6ac	4.52	259.8 ± 53.3	0.585	5
6ad	0.74	160900		1
6ae	4.21	756.8 ± 0.1	0.210	1
6af	5.01	51.6 ± 8.5	1.288	4
6ag	2.45	>500		1
6ah	9.96	inactive		1

[a] Octanol/water partition coefficients. [b] $C = IC_{50} \times 10^{-3}$ [c] From ref 13. [d] From ref 5d.

The parabolic relationship between activity (log $1/C$) and hydrophobicity (octanol/water partition coefficient), expressed as log P as shown in Figure 2, was statistically significant (eq 1).

Table 6. Human Platelet Aggregation Inhibitory Activities and Partion Coefficients of Type **7** Compounds

compd no.	log P^a	IC_{50} (μM ± SE)	n
7a	9.06	16.0 ± 2.2	5
7b	9.578	20.9 ± 3.5	5
7c	10.096	23.8 ± 4.5	6
7d	10.096	22.9 ± 4.9	6
7e	11.322	25.9 ± 3.8	6
7f	4.629	84.6 ±19.6	4
7g	2.636	2046	1

[a]Octanol/water partition coefficients.

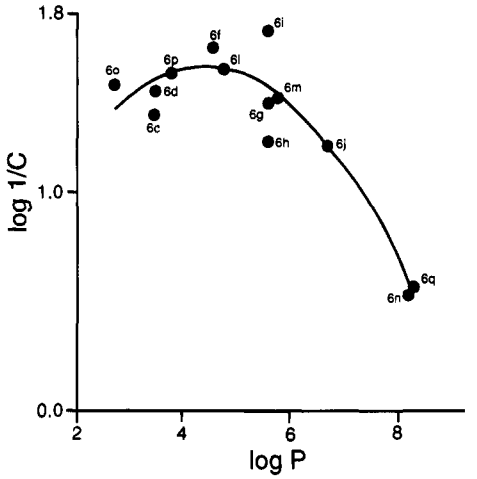


Figure 2. Relationship between hydrophobicity (log P; P = octanol/water partition coefficient) of bisnipecotamidoaralkanes (type **6**) and human platelet aggregation−inhibitory activity (log $1/C$; $C = IC_{50} \times 10^{-3}$; μM compound concentration inhibiting ADP-induced aggregation by 50%).

$$\log 1/C = 0.632 (\log P) - 0.073 (\log P)^2 + 0.190 \quad (1)$$

$$n = 13; \quad r = 0.944; s = 0.155; F_{\alpha=0.01} = 41.45$$

Thus, the single parameter log P accounts for 89% ($r^2 = 0.892$) variance in the activities of nipecotamides. The optimum log P of 4.5 was also found earlier for some type **6** compounds.[13]

Modification of the Piperidine Ring 3-Substituent. A 3-substituted piperidine ring appeared necessary for activity since compounds with unsubstituted piperidine ring were inactive. Thus **6a**, a 3-unsubstituted piperidine analog of **6c**, has IC_{50} 8463 μM compared to 44.5 μM of the latter (*cf.* ref 12). Branching of one or both of the amide N substituents resulted in a loss of potency. Thus, **6e** (NCH_3, *i*-Pr) was less potent (IC_{50} 144.3 μM) than **6d** (NCH_3, *n*-Pr, IC_{50} 34.6 μM). Also, **6h** (N-*i*-Pr_2) was less active (IC_{50} 58.8 μM) than **6g** (N-*n*-Pr_2, IC_{50} 39.3 μM).

A 3,4,5-trimethoxybenzyl group was substituted on the 3-amide N with a view to mimic the 3,4,5-trimethoxybenzyl moiety of tretoquinol (trimetoquinol), a potent antiplatelet compound.[16] The resultant compound **6ab** was only moderately active (IC_{50} 134.2 μM) compared to its analog **6k** (IC_{50} 53.6 μM), which is structurally identical, but without the methoxy substituents.

It was demonstrated earlier that reduction of the 3-amide group of **6c** to a CH_2N resulted in a 35-fold attenuation of potency indicating the requirement of an amide O atom on the piperidine 3-substituent.[13] Compound **6x** which has an α-hydroxyethyl substituent on the 3 position showed low activity (IC_{50} 589.4 μM). Also, **6ad** which carries a reverse amide attached to a 3-methylene substituent has very feeble activity (IC_{50} 160 900 μM). Although **6ah** does not really belong to type **6**, it is a nipecotamide carrying a reverse amide function on the 3 position. It has very low activity suggesting the requirement that a 3-amide C should be directly linked to the heterocyclic ring. This finding is further substantiated by the fact that **6b**, which has a 3-methyl substituent, has very feeble activity (IC_{50} >500 μM) despite a good degree of lipophilicity (log P 4.604). It has been postulated that the 3-amide O atoms of nipecotamides form H-bonds with the 3-OH group of phosphatidylinositol 4,5-bisphosphate (of the platelet's inner leaflet).[13] The significance of electronegativity of the 3-amide O in this interaction is exemplified by the relative activities of **6c** and **6ae**. The structure of **6ae** contains a 3-C(=O)OEt group. Although the lipophilicity of this molecule (log P 4.21) is near optimum, it is much less potent (IC_{50} 756.8 μM) than the corresponding *N,N*-diethylamide **6c**. MOPAC charges calculated using the AM1 method are −0.377 and −0.374 au, respectively, for the two amide O atoms in *R,S*-**6c** *vs* −0.355 and −0.356 au for the two 3-C(=O) oxygens, respectively, in *R,S*-**6ae**.

Cyclization of one of the 3-amide *N*-substituents resulted in a small increase in potency as in the case of **6c** (N-Et_2; IC_{50} 44.5 μM) *vs* **6o** (3-pyrrolidinyl; IC_{50} 32.7 μM).

Piperidine-3-thioamides were synthesized with the goal of improving potency by way of increasing lipophilicity. However, the resultant compound **6af** (log P 5.01; IC_{50} 51.6 μM) was somewhat less active than the corresponding amide **6c** (log P 3.48; IC_{50} 44.5 μM).

Other Modifications of the Piperidine Ring. 3,5-Disubstitution as in **6w** (IC_{50} >500 μM) led to a decline in activity compared to 3-substituted analogs (**6c**), suggesting probable steric hindrance in the antagonist's binding to sites in/on the platelets.

Based on the duration of action of **6c** *ex vivo* in the dog, and on preliminary biotransformation experiments, it was suspected that the 2-CH_2 group on the piperidine ring is oxidized *in vivo* (Gollamudi *et al.*, unpublished observations). Compound **6ac**, a 2-methyl analog of **6c** synthesized with a view to extend the duration of action, was only one-sixth as active as **6c**, indicating that the 2 position of the piperidine ring should be unsubstituted for optimum activity. As noted above (**6w**), the 5 position also should be unsubstituted for optimum activity.

Modifications of the Connecting Bridge. The two ring N atoms in the more active type **6** compounds are connected by a xylylene link, and are separated by ~7 Å.[17] For example, the interatomic distances in the more active compounds are **6i** 7.006 Å, **6f** 7.042 Å, **6l** 7.086 Å, **6p** 6.932 Å, **6o** 6.960 Å, **6d** 6.989 Å, **6m** 7.154 Å, **6g** 7.045 Å, and **6c** 6.925 Å. Thus, the optimal separation distance between the two ring nitrogens appears to be ~7Å. For example, the only difference between **6c** and **6z** is that the latter has an additional benzene ring connecting the two nipecotamide groups; i.e., the ring nitrogens in **6c** are connected by a *p*-xylylene link, whereas in **6z** they are connected by a 4,4′-bis(methylene)-1,1′-biphenyl moiety. The two ring nitrogens in **6z** are separated by 11.62 Å, and although the log P (5.43) is within the desirable range, the activity of this

compound (IC_{50} 478.5 μM), however, was much lower (1/11th) than that of **6c**. To the contrary, **6aa** serves as an example of a bisnipecotamide with a shorter interatomic distance (6.178 Å). In this analog, the connecting bridge is 2,2′-bis(methylene)-1,1′-biphenyl. Although the log *P* of **6aa** is 5.43, the activity was lower (IC_{50} 353.5 μM) than that of **6c**. These examples suggest that the separation distance between the two ring nitrogens should be ~7 Å for favorable interaction with complementary anionic platelet sites. Also, these platelet sites may be separated by a hydrophobic well capable of accommodating aromatic groups and aliphatic hydrocarbon chains. A separation distance of ~8 Å has been proposed for optimum interaction with platelet aggregation–inhibitory specific target sites.[12,15] It has been suggested that the lipophilic nipecotamides can penetrate the lipid bilayer of platelets, and at the pH of the cytosol, the tertiary piperidine N is protonated. The latter is capable of interacting with and neutralizing the charge density anionic phosphoinositides of the inner leaflet, thereby impeding its hydrolysis to inositol 1,4,5-trisphosphate, an important second messenger in signal transduction in platelets.[4-6,13] In this context, the basicity of the piperidine ring's tertiary N atoms appears to be of importance. 1,4-Bis[3-(*N*,*N*-diethylcarbamoyl)piperidino]benzenedicarboxamide constitutes an appropriate example. In this molecule, the two methylene groups of **6c** are replaced by two carbonyl groups which render the adjacent N atoms less electronegative, and consequently the compound is much less active (IC_{50} 20 850 μM) than **6c**.[18a]

It appears that an aromatic ring connecting the two nipecotoyl moieties confers greater antiplatelet potency than aliphatic or carbocyclic groups. For instance, replacement of the benzene ring of **6c** with a cyclohexyl moiety as in **6y** resulted in a precipitous decline in antiplatelet activity (IC_{50} > 1000 μM) despite a log *P* of 4.57 and a separation distance of 7.683 Å. Also, replacement of the xylylene link of **6c** by $-(CH_2)_{10}-$ as in **6u** (piperidine N separation distance 6.02–14.07 Å) led to lower activity (IC_{50} 207.0 μM) although the log *P* was 5.92. Van der Waal's forces and π interactions may be operative between nipecotamides and target sites on/in the platelets. Also, the greater flexibility variance in the interatomic distances between the ring nitrogens of bisnipecotoylalkanes, compared to that of -aralkanes is known to be a contributing factor in the former's weaker potency.[12,15]

Among the bis nipecotoylalkanes, 12 methylene units connecting the two rings as in **6v** afforded greatest activity (IC_{50} 85.3 μM), followed by 10 (**6u**, IC_{50} 207.0 μM) and 8 (**6t**, IC_{50} 213.9 μM) $-CH_2-$ units. The distances between the ring nitrogens in these compounds/their flexibility variances and their (log *P* values) are: **6v**, 8.715/7.548–16.263 Å (6.96); **6u**, 8.050/6.020–14.07 Å (5.92); and **6t**, 5.421/6.075–11.496 Å (4.89). Clearly, all three have the potential for a 2-point attachment with target sites, and although the flexibility variance in **6v** is the highest, it is the most potent of the bisnipecotoylalkanes tested. It is possible that the hydrophocity of these compounds bears a greater influence on their activities than the interatomic distances between the piperidine nitrogens. Also, the parabolic relationship between log *P* and antiplatelet activities noticed earlier with bisnipecotoylaralkanes (eq 1) does not appear to be operative in this series.

Type 5 Compounds. The most potent among the 1-alkylnipecotamides and related molecules (Table 4) is **5a** (IC_{50} 241.9 μM; log *P* 5.76). To improve on the activity of this molecule, the 1-decyl substituent was replaced by an aralkyl (benzyl) group. The resulting compound **5h** was inactive (IC_{50} 7399 μM; log *P* 2.75). To enhance the activity of the latter, electron-attracting and electron-releasing groups with lipophilic potential were substituted on the aromatic ring. Among the monochloro analogs (log *P* 3.49), the order of potency (IC_{50}, μM) was *m*-Cl (**5d**, 1652.8) > *p*-Cl (**5b**, 216.6) > o-Cl (**5f**, 2805.5). The *p*-*tert*-butylbenzyl derivative (**5e**) was inactive at 200 μM despite an increase in lipophilicity (log *P* 4.826), suggesting that electronic factors probably have minimal influence on activity. Although it is possible that substituents in the *o*- and *p*-positions offer steric hindrance for a proper binding of the nipecotamide to a critical platelet site, it may not be appropriate to draw conclusions from the limited number of type **5** structures studied. Also, the very high concentrations used may cause other nonspecific interactions. On the basis of preliminary results using a mouse thrombosis model,[10] it appears that these halogen derivatives (**5b**,**d**,**f**) are quite active *in vivo*, although they were inactive *in vitro* (Han, Lawrence, and Gollamudi, unpublished results).

With a view to increase the lipophilicity of **5a** (log *P* 5.76), its thioamide, **5c**, (log *P* 6.53) was synthesized. The activity (IC_{50} 358.4 μM) of the latter, however, was less than that of **5a** (IC_{50} 241.9 μM), emphasizing the need for optimizing rather than increasing lipophilicity. Further, among compounds possessing the same degree of lipophilicity, steric effects also appear to be operative in determining potency.

Type 7 Compounds. Among the bis(*N*-alkylnipecotoyl)piperazines and related compounds (**7a**–**d**) evaluated, **7a** was the most active one (Table 6). Any modification of this structure resulted in a loss of potency. These compounds are highly lipophilic (log *P* > 9.0) and consequently are poorly water-soluble, posing formulation problems which precluded further optimization of structure. With a view to rendering them water-soluble, a phthalimidoethyl group was substituted on the ring N in place of the *n*-decyl group of **7a**. While the resultant compound **7g** was water-soluble, its activity was quite low (IC_{50} > 2046 μM). Mono- and disubstitution of the piperazine ring as in **7b**, **c**, and **d** led to a decline in potency. Increasing the heterocyclic ring size to that of a homopiperazine (**7f**) also resulted in a loss of activity.

The separation distance of the two piperidine N atoms in **7a**, the most active compound in this series (IC_{50} 16.0 μM), is 9.8 Å, and that of **7e** (IC_{50} 25.9 μM) is 12 Å. These are much greater than the 7 Å occurring in type **6** compounds. Clearly, the interactions between tertiary N atoms and phosphoinositides discussed earlier in the context of type **6** molecules appear to be operative by other, as yet unknown mechanisms in the case of type **7** nipecotamides.

Other Structural Modifications. 1-[2-(Acetyloxy)benzoyl]-3-(*N*,*N*-diethylcarbamoyl)piperidine (**ASP-1**) incorporates the structural features of **5c** as well as those of aspirin. **ASP-1** was inactive *in vitro* (IC_{50} >

40 000 μM with ADP and >1567 μM with collagen) but was active *in vivo* (ED_{50} 450 mg/kg) in protecting mice from thromboembolic death induced by intravenous collagen + epinephrine.[18b] It is suggested that *in vivo*, the acetyl group of **ASP-1** may be hydrolyzed and transferred to the platelet (cyclooxygenase) in a manner similar to that of aspirin.

In conclusion, we have prepared and evaluated 48 nipecotamides of 3 structural types, carrying alkyl or aralkyl substituents on the piperidine N atom. Bisnipecotoylaralkanes were more potent than their monoanalogs. In addition to their enantiospecific actions noted earlier,[13] molecular determinants of activity included steric factors and lipophilicity. Chiral resolution and asymmetric synthesis of the more active molecules like, for example, **6d** and **6i**, aimed at improving their therapeutic index, are in progress. Nipecotamides of types **5**, **6**, and **7** were reported to inhibit aggregation induced by a variety of agonists. Compound **6c** was reported to completely inhibit ADP-induced aggregation, preventing shape change.[21a] The relative potencies of several nipecotamide inhibitors varied with each agonist. Investigations into the structure–activity relationships using ADP,[13] collagen,[5] and epinephrine[7] in particular suggest that these inhibitors act at multiple platelet sites.

It has been postulated that the surface-active nipecotamides can penetrate the lipid bilayer of the platelet membrane as unionized bases.[6,21b] Subsequent to protonation at the pH of the cytosol, the cationic piperidine N neutralizes the platelet membrane's (anionic) phosphoinositides, thereby reducing their response-sensitivity to phospholipase C (PLC)-mediated hydrolysis to inositol 1,4,5-trisphosphate (IP_3),[7,21c,d] a mediator of the discharge of cytosolic ionized calcium ($[Ca^{2+}]_i$).[21e,f] Further, by enhancing the integrity of the membrane complexes sequestering Ca^{2+} (*e.g.*, the dense tubular system) the compounds could block Ca^{2+} release into the cytosol.[21d] The inhibition of the rise in agonist-induced platelet $[Ca^{2+}]_i$ by nipecotamides was demonstrated recently.[5d] Results from preliminary experiments suggest that in the prostaglandin (PG)-dependent pathway, nipecotamides may act at some postreceptor signal transduction event(s) leading to the discharge of $[Ca^{2+}]_i$. As with many therapeutic agents, the mechanism of antiplatelet action of nipecotamides is not clearly known. Investigations addressing this aspect are in progress.

Experimental Section

Melting points were determined on an Electrothermal 9200 apparatus and are uncorrected. Elemental analyses of the synthetic compounds were performed at Atlantic Microlab Inc., Norcross, GA. IR spectra were obtained using a Perkin-Elmer 2000 FT IR instrument. ^{1}H-NMR spectra were recorded on a Bruker ARX-300 FT spectrometer. Fast atom bombardment (FAB) mass spectra were recorded on a VG Autospec Q instrumment. Log *P* values were calculated using the PROLOG P program of CompuDrug Inc. Molecular modeling was performed using the software package SYBYL (Tripos Associates, version 6.0) on a Silicon graphics terminal.

Chemistry. Previously reported methodologies were appropriately modified and adapted for the synthesis of nipecotamides (Scheme 1) discussed here.[19]

Type 5 Compounds: Synthesis of 1-*tert*-Butyl-3-(*N*,*N*-diethylcarbamoyl)piperidine Hydrobromide (5e). Step 1. 4-*tert*-Butylbenzyl bromide (22.7 g, 0.10 mmol) dissolved in 200 mL acetone was added dropwise to a stirred solution of *N*,*N*-diethylnicotinamide (17.8 g, 0.10 mmol) in 250 mL of absolute ethanol maintained below 25 °C. The mixture was gradually heated and refluxed 29 h. The solvent was then removed and the residue was recrystallized from CH_3CN:EtOAc (2:3) to obtain **5e**$_i$ (28.3 g, mp 196.1–197.7 °C): MS, EI *m/z* 326 [MH^+]; fragmentation consistent with proposed structure.

Step 2. Catalytic reduction (1.0 g of PtO_2) of 20.3 g (0.05 mmol) of **5e**$_i$ in 250 mL of absolute ethanol (ambient temperature, 60 psi), followed by recrystallization of the product from CH_3CN:EtOAc (1:8) yielded 5.0 g of **5e**: mp 218.7–219.6 °C; MS *m/z* 331 [MH^+]; fragmentation consistent with proposed structure; ^{1}HNMR (D_2O) δ ppm 7.5 (s, 4H, Ar*H*), 4.20–4.45 (m, 1H, C*H*CON), 3.00–3.65 (m, 8H, CON(CH_2CH_3)$_2$ and piperidine CH_2NCH_2), 1.35 (s, 9H, C(CH_3)$_3$, 1.10 (t, 6H, (CH_2CH_3)$_2$). Anal. ($C_{21}H_{35}N_2OBr$) C, H, N, Br.

Other type **5** compounds, **5b**, **5d**, and **5f** were synthesized following the same outline as described above.

Type 6 Compounds: Synthesis of α,α′-Bis[3-(*N*-acetylaminomethyl)piperidino]-*p*-xylene Dihydrobromide (6ad). Step 1. Acetyl chloride (38.55 g, 0.491 mol) was added dropwise to a cooled (20–25 °C) mixture of 3-(aminomethyl)-pyridine (53.1 g, 0.491 mol) and triethylamine (50.95 g, 0.504 mol) in 50 mL of methylene chloride. The reaction mixture was refluxed for 5 h and cooled. After addition of 150 mL of water, the product was extracted with 4 × 200 mL of EtOAc. The extract was dried (anhydrous $MgSO_4$) and the solvent evaporated to yield the crude product which upon distillation (184 °C, 1.5 mm) gave 7.5 g of 3-[(*N*-acetylamino)methyl]-pyridine (**6ad**$_i$).

Step 2. **6ad**$_i$ (7.0 g, 0.0466 mol) in 30 mL of ethanol was condensed with α,α′-dibromo-*p*-xylene (5.9 g, 0.0225 mol) in 57 mL of acetone by refluxing for 4 h to form α,α′-bis[3-[(*N*-acetylamino)methyl]pyridiniumyl]-*p*-xylene dibromide (**6ad**$_{ii}$), which was recrystallized from ethanol:methanol (6:1) to yield 9.8 g of pure **6ad**$_{ii}$, mp 236.7–237.9 °C.

Step 3. Catalytic reduction (0.5 g of PtO_2, 60–65 psi, 25 °C) of **6ad**$_{ii}$ (9.0 g, 0.16 mol) in ethanol (50 mL) and water (130 mL) and recrystallization of the product from MeOH:EtOAc (3:1) gave 0.5 g of **6ad**, mp 279.1–279.8 °C.

Other type **6** compounds were synthesized via Scheme 1 according to the procedure described by Feng et al.[13]

Type 7 Compounds: Synthesis of *N*,*N*′-Bis[1-[2-(phthalimido)ethyl]nipecotoyl]piperazine Dihydrobromide (7g). Step 1. Thionyl chloride (71.38 g, 0.6 mol) was added dropwise to a cold (<25 °C) stirred mixture of nicotinic acid (73.87 g, 0.6 mol) and pyridine (97 mL, 1.2 mol) in 300 mL of toluene. After the reaction mixture was heated to and maintained at 90–95 °C for 1 h, piperazine (25.8 g, 0.3 mol) in hot toluene (300 mL) was added dropwise. The reaction mixture was stirred for an additional 4 h, after which the toluene layer was separated and washed with 200 mL of water. The aqueous extract was combined with the aqueous layer from the reaction mixture. The pH was adjusted to 9.0 with 29% aqueous Na_2CO_3, and the amide was extracted with 6 × 200 mL of chloroform. The extract was dried (anhydrous $MgSO_4$), filtered, and concentrated to 300 mL and cooled. The separated solid product was filtered and dried to yield 38.8 g (0.131 mol) of *N*,*N*′-bis(nicotinoyl)piperazine dibromide (**7g**$_i$).

Step 2. To a stirred solution of **7g**$_i$ (25.0 g, 0.084 mol) in 350 mL of absolute ethanol was added dropwise *N*-(2-bromoethyl)pthalimide (44.38 g, 0.175 mol) dissolved in hot acetone (150 mL). After refluxing for 240 h, the solid reaction product was separated and washed with absolute ethanol to yield 2.9 g of *N*,*N*′-bis[1-(2-phthalimidoethyl)nicotinoyl]piperazine dibromide (**7g**$_{ii}$).

Step 3. Catalytic reduction (0.5 g of PtO_2) of 2.2 g (0.003 mol) of **7g**$_{ii}$ in 100 mL of methanol and 70 mL of water (50 °C, 60–65 psi) followed by recrystallization from methanol afforded 100 mg of **7g**.

***N*,*N*′-Bis(1-decylnipecotoyl)-2-methylpiperazine (7b)** was prepared by a modification of the procedure reported by Badgett et al.[20] (*cf.* ref 6).

Step 1. To a cooled (<30 °C), stirred solution of nicotinic acid (73.9 g, 0.6 mol) in 300 mL of toluene and 97.1 mL of pyridine was added dropwise thionyl chloride (71.4 g, 0.6 mol).

The temperature was then gradually raised to 95–98 °C. After 1 h, 2-methylpiperazine (30.1 g, 0.3 mol) in 300 mL of toluene was added from a steam-jacketed dropping funnel, and the reaction was continued at 95–98 °C for 4 h. The solid reaction product was dissolved in water, the pH was adjusted to 9.0 with 29% aq. Na_2CO_3, and the reaction product was extracted with $CHCl_3$. Evaporation of the solvent yielded an oily residue which was dissolved in hot EtOAc. Upon cooling, *N,N'*-bis(nicotinoyl)-2-methylpiperazine (**7b$_i$**) separated as yellow crystals (52.7 g), mp 108–109 °C. Anal. ($C_{17}H_{18}N_4O_2$) C, H, N.

Step 2. To a stirred, heated (105 °C) solution of **7b$_i$** (15.5 g, 0.05 mol) in 270 mL of dioxane was added dropwise 1-iododecane (29.5g, 0.1 mol), and the reaction was continued for 81 h. The reaction product (37.1 g) was recrystallized from 270 mL of absolute ethanol to give 23.6 g of *N,N'*-bis(1-decylnicotinoyl)-2-methylpiperazine diiodide (**7b$_{ii}$**, 23.6 g), mp 155–156 °C. Anal. ($C_{37}H_{60}N_4O_2I_2$) C, H, N, I.

Step 3. Hydrogenation (PtO_2/H_2, 59.4–60.5 psi, 2 h) of **7b$_{ii}$** (13.5 g in 270 mL of 75% aq. ethanol) followed by recrystallization from ethyl acetate afforded 11.6 g of pure **7b**, mp 212–213 °C. Anal. ($C_{37}H_{72}N_4O_2I_2$) C, H, N, I.

Other type **7** compounds, **7c**, **7d**, and **7a**,[6] were synthesized using the procedure exemplified by **7b** above.

Measurement of Platelet Aggregation–Inhibitory Activity. Blood was obtained from male and female donors, 25–35 years of age, who had fasted overnight and affirmed abstinence from all medications, alcohol, caffeine, and tobacco for at least 1 week prior to donation. Plasticware was used to handle blood and plasma except for siliconized glass cuvettes and siliconized metal stir bars. Test compounds were dissolved in redistilled water (**5a–h**, **6a–b**, **6d**, **6g–v**, **6x**, **6z**, **6ab–ah**, **7f–g**), redistilled 95% ethanol (**7a–e**), or redistilled 95% ethanol:water [1:1] (**6a**, **6e–f**). The minimal concentration of adenosine diphosphate (ADP) eliciting full biphasic aggregation response (9.6 μM ± 0.9 SE for 117 samples) was established using platelet-rich plasma (PRP) from each donor prior to the determination of each test compound's inhibitory potency. PRP was prepared by centrifugation of citrated blood (blood: 3.2% sodium citrate = 8:1) at 120*g*, 15 min, 23 °C and recovering the upper layer. Platelet poor plasma (PPP) was obtained similarly by centrifuging at 1100*g* for 18 min. The platelet count of PRP was adjusted to 250 000–300 000/mm^3 using autologous PPP. The plasma was gassed with 5% CO_2 + 95% air (v/v), capped, and maintained at 37 °C. It was then transferred (450 μL) to cuvettes with stir bars in a Marster's constant temperature (37 °C) block. Bubbles were rapidly removed from the PRP followed by capping the cuvettes with Parafilm to prevent CO_2 loss and pH rise. The cuvettes were transferred to a Payton Associates dual channel aggregometer equipped with a Fisher dual channel omniscribe recorder, and after 15 s of stirring at 1100 rpm, the test compound solution (1.0 μL) or an equal volume of the appropriate solvent was added to the PRP. Fifteen seconds after the addition of the test compound, the cuvette was transferred back to the constant temperature block. After 1 min 45 s, the cuvette was returned to the aggregometer. After 4 min of incubation with the test compound, 50 μL of ADP was added. Changes in light transmission were recorded for another 5.5 min. The "control" cuvette was initiated 1 min after the "treated" cuvette and followed the same sequence of events.

The percent inhibition of aggregation (*I*%) was calculated as the difference between maximum "control" and "treated" pen responses expressed as a percentage of the maximum control response. The IC_{50} for aggregation (concentration of test compound causing 50% inhibition of aggregation) was determined by the linear regression (employing Lotus 123 Release 3.0) of *I*% on log molar concentration of the test compound.

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