Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry 11 (2003) 1439–1450

BIOORGANIC & MEDICINAL CHEMISTRY

Investigation of Platelet Aggregation Inhibitory Activity by Phenyl Amides and Esters of Piperidinecarboxylic Acids

Modesto de Candia,[a] Luciana Summo,[a,†] Antonio Carrieri,[a] Cosimo Altomare,[a,*] Adele Nardecchia,[b] Saverio Cellamare[c] and Angelo Carotti[a]

[a]*Dipartimento Farmaco-chimico, Università degli Studi, Via Orabona 4, 70125, Bari, Italy*
[b]*Dipartimento di Medicina e Oncologia, Ospedale Policlinico, P.zza G. Cesare 1, 70125, Bari, Italy*
[c]*Dipartimento di Scienze del Farmaco, Università 'G. D'Annunzio', Via dei Vestini, 66100, Chieti, Italy*

Received 2 August 2002; accepted 11 November 2002

Abstract—A series of anilides and phenyl esters of piperidine-3-carboxylic acid (nipecotic acid) were synthesized and tested for the ability to inhibit aggregation of human platelet rich-plasma triggered by adenosine 5′-diphosphate (ADP) and adrenaline. As a rule, amides were about two times more active than the corresponding esters, and derivatives bearing substituents at the *para* position of the phenyl ring were significantly more active than the *meta*-substituted ones. Among the tested compounds, 4-hexyloxyanilide of nipecotic acid (**18a**) was found to be the most active one, its IC_{50} value being close to that of the most active bis-3-carbamoyl-piperidines reported in literature (ca. 40 μM) and aspirin (ca. 60 μM) in ADP- and adrenaline-induced aggregation, respectively. Compared with the isomeric 4-hexyloxyanilides of piperidine-2-carboxylic (pipecolinic) and piperidine-4-carboxylic (isonipecotic) acids, compound **18a** showed higher activity, and a Hansch-type quantitative structure–activity relationship (QSAR) study highlighted lipophilicity and increase in electron density of the phenyl ring as the properties which mainly increase the antiplatelet activity ($r^2 = 0.74$, $q^2 = 0.64$). The interaction of nipecotoyl anilides with phosphatidylinositol, a major component of the inner layer of the platelet membranes, was investigated by means of flexible docking calculation methods to give an account of a key event underlying their biological action.

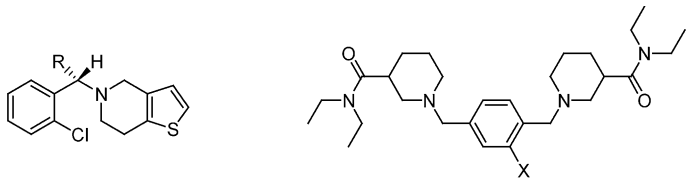
Introduction

Thrombosis and thromboembolism are major causes of morbidity and mortality.[1] Platelet aggregation is a central step in the formation of intravascular thrombi, and deposition of platelets is also involved in the formation of atherosclerotic plaques.[1,2] When vascular endothelium is damaged, the subendothelial matrix exposes adhesive proteins, such as collagen, and the contact with them activates the platelets. As a consequence the platelets change their discoidal shape, form a network in the site of injury and release from their dense granules a number of physiological proaggregatory agonists, such as adenosine-5′-diphosphate (ADP), platelet activating factor (PAF), serotonin, thromboxane A_2 (TxA_2) and cathecolamines.[3–6] These agonists can activate the surrounding platelets leading to thrombus formation. Although it is a weak platelet activator, ADP plays a relevant role in platelet function. It can trigger platelet activation, which is mediated by three purinergic receptors (P_2Y, P_2T, P_2X) showing distinct specificity, and is responsible for the secondary wave of platelet aggregation, followed by ADP release from dense granules.[7–9]

Antiplatelet agents, such as aspirin, dipyridamole, thienopyridines, and platelet glycoprotein IIb/IIIa antagonists have amply demonstrated their utility in preventing and treating coronary artery thrombosis.[10,11] In particular, ticlopidine and clopidogrel, active in *S*-configuration (Fig. 1), both belonging to the thienopyridine class, have been increasingly used to prevent ischemic events. Moreover, their association with aspirin are effective in suppressing thrombotic complications after coronary stenting and in other conditions in which patients are at high risk of atherothrombotic events.[12,13] Despite their benefits, administration of thienopyridines can in certain cases be associated with several undesired side effects (e.g., neutropenia, agranulocytosis, gastrointestinal toxicity),[12] whereas a problem

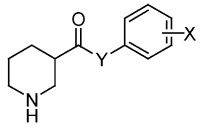
*Corresponding author. Tel.: +39-080-544-2781; fax: +39-080-544-2230; e-mail: altomare@farmchim.uniba.it
†Present address: Berlin Chemie AG, Menarini Group, Glienicker weg 125, 12489 Berlin, Germany.

0968-0896/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
PII: S0968-0896(02)00599-0



R = H, Ticlopidine
R = $COOCH_3$, Clopidogrel

1a, X = O-nC_6H_{13}
1b, X = NH_2



Y = O, NH, NCH_3, X = see Table 1
2-22

Figure 1. Structures of antithrombotic thienopyridines (ticlopidine and clopidogrel), literature carbamoylpiperidines (**1**), and general stucture of the piperidine-3-carboxylic acid derivatives examined in this paper (**2**–**22**).

in clinical use of all the currently available antiplatelet agents is the interference of physiological platelet function in haemostasis.[14] Therefore, the development of antiplatelet agents, showing limited side effects, is still a relevant goal of the pharmaceutical research.

Among the numerous compounds reported in literature, amides of piperidine-3-carboxylic acid (nipecotamides), namely α,α′-bis[3-(*N*,*N*-dialkylcarbamoyl)piperidino]-*p*-xylenes **1** (Fig. 1), found to inhibit in vitro human platelet aggregation induced by ADP, collagen, thrombin, adrenaline, and the stable TxA_2-mimetic U46619, and to be effective in in vivo thrombosis models,[15–17] attracted our attention.

Bis-3-carbamoylpiperidine derivatives bearing *n*-hexyloxy (**1a**) or amino (**1b**) substituents at C-2′ of the phenyl group in the xylene bridge have been found to be among the most active, whereas from structure–activity studies[15,17] it had been inferred that double cationic head, amide carbonyl, appropriate stereochemistry of the C-3, and a log P value of about 4.5 have to be considered relevant molecular determinants of the antiplatelet activity. A hypothesis on the mechanism of action of nipecotamides has been suggested based on several studies carried out by Gollamudi and coworkers.[15,17–29] Due to their lipophilicity, and surface activity, nipecotamides can penetrate the platelet membranes and interact with anionic phospholipids (mainly phosphatidylinositol, PI, and phosphatidylserine, PS), rendering them resistant to hydrolysis catalyzed by phospholipase-C to the second messengers inositol 1,4,5-triphosphate (IP_3) and *s*,*n*-1,2-diacylglycerol (DAG). Nipecotamides inhibit the phosphoinositide turnover as well. As a consequence, the levels of IP_3 and of cytosolic Ca^{2+} concentrations, necessary for myosin phosphorylation and subsequent platelet activation, are reduced.

To the best of our knowledge, literature is lacking of information on the effects on platelet aggregation of simple aryl amides of piperidine-3-carboxylic acid, which in contrast should allow one to challenge the hypothesis of the need of a double amonium head and to modulate the lipophilicity of the phenyl substituents. As a part of our ongoing research aimed at developing new potential antithrombotic drugs,[30,31] we screened a series of derivatives structurally simpler than compounds **1** to gain insights into the minimal structural features necessary for antiplatelet activity of nipecotamides. Thus, we synthesized and tested for their effects on the human platelet aggregation (ADP and adrenaline were chosen among the several known triggers) a series of nipecotic acid anilide congeners, and a number of phenyl esters as well, bearing in *meta* and *para* positions of the phenyl group substituents selected according to the Hansch QSAR analysis principles. A comparison of the most active nipecotic acid derivatives with their isomers obtained from pipecolinic (piperidine-2-carboxylic) and isonipecotic (piperidine-4-carboxylic) acids was also undertaken. Herein, we report on structure–activity relationships of these compounds.

Chemistry

The phenyl piperidine-3-carboxamides (Table 1, **2a**–**20a**) and anilides **23a** and **24a** (Table 2) were synthesized using known methods according to the pathway shown in Scheme 1.

1-(*Tert*-butyloxycarbonyl)-piperidine carboxylic acids, prepared by a reported method,[32] were condensed with *para* or *meta* substituted anilines, using dicyclohexylcarbodiimmide (DCC) and 1-hydroxybenzotriazole hydrate (BtOH) as coupling reagents in dry dichloromethane. *N*-[4-(hexyloxy)phenyl]-*N*-methylamine, necessary to synthesize derivative **19a**, was obtained through a one-pot reductive methylation of *p*-hexyloxy aniline in the presence of aqueous formaldheyde (10% Pd/C as catalyst). The nitroanilides **6a** and **7a** were prepared in better yields by reacting the *N*-BOC-nipecotoyl chloride with *meta*- and *para*-nitroaniline, respectively. *N*-BOC-*p*-nitrophenyl amide (**7a**) was reduced by catalytic

Table 1. Antiplatelet activity and lipophilicity of X-substituted phenyl esters and anilides of nipecotic acid

Compd	Y	X	IC_{50} (μM) or% inhibition at 100 μM[a]		log P[b]
			ADP	Adrenaline	
Control			15±1%	15±1%	
2a	NH	H	200	4±2%	1.53
2b	O	H	570	0%	1.78
3a	NH	3-Br	0%		2.65
4a	NH	4-Br	162	39±13%**	2.65
4b	O	4-Br	245		2.78
5a	NH	4-Cl	10±1%		2.50
6a	NH	3-NO_2	0%		1.82
7a	NH	4-NO_2	28±6%**	12±7%	1.82
7b	O	4-NO_2	10±1%		1.82
8a	NH	4-NH_2	0%		0.30
9a	NH	4-CF_3	372		2.86
10a	NH	4-CN	0%		1.56
11a	NH	4-CH_3	270		2.03
12a	NH	3-OCH_3	0%		1.60
13a	NH	4-OCH_3	218	4±2%	1.60
13b	O	4-OCH_3	5±1%		1.87
14a	NH	4-$NHSO_2CH_3$	23±3%*		0.34
15a	NH	4-$COCH_3$	0%		1.44
16a	NH	4-$N(CH_3)_2$	0%		1.69
17a	NH	4-$C(CH_3)_3$	269	5±1%	3.35
17b	O	4-$C(CH_3)_3$	175		3.61
18a	NH	4-OnC_6H_{13}	45.1	66.0	4.25
18b	O	4-OnC_6H_{13}	100	38±5%***	4.52
19a[c]	NCH_3	4-OnC_6H_{13}	120[d]	70.2	4.31
20a	NH	4-nC_7H_{15}	102		5.20
21b	O	4-I	214		3.04
22b	O	4-OCH_2Ph	235		3.64
1a			38.7		
ASA				59.1	

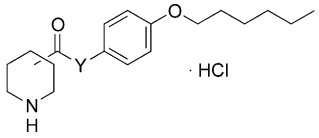
[a]Platelet-rich plasma (PRP) was pre-incubated with the test compound or with dmso (0.5% v/v, control) at 37 °C for 5 min. The inducer, ADP (10 μM) or adrenaline (10 μM), was then added. Activity is expressed as IC_{50} or percentage of the inhibitory response for less active compounds at 100 μM concentration (means±SEM, $n \geq 3$); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ significantly different from control.
[b]*n*-Octanol-water partition coefficient (log P) calculated by CLOG P software vers. 4.0 (Biobyte, Claremont, CA, USA).
[c]Maleate salt.
[d]Value obtained by extrapolation; 100 μM was the maximum concentration tested.

hydrogenation to *p*-aminophenyl amide (**8a**), that in turn was reacted with methanesulfonyl chloride to give the *N*-BOC derivative of compound **14a**, and transformed into the dimethylamino congener **16a** through a one-pot reductive methylation with aqueous formaldheyde. Deprotection of the *N*-BOC intermediates with HCl gas yielded the amide derivatives as hydrochloride salts. Physical and spectral data of the newly synthesized compounds are reported in Table 3. The phenyl esters of piperidinecarboxylic acid tested in this study (**2b**, **4b**, **7b**, **13b**, **17b**, **18b**, **21b**–**24b**) were synthesized using our previously reported procedures.[33]

Results and Discussion

The nipecotic acid derivatives were tested as antiplatelet agents by measuring their inhibitory actions on the in vitro aggregation of human platelet-rich plasma (PRP) triggered by ADP and adrenaline, both at 10 μM concentrations, by using a turbidimetric method.[19,30,34,35] The aggregation tracing relative to the controls revealed a typical biphasic aggregation with both agonists. In a preliminary screening, carried out at a dose of 100 μM, a number of the test compounds showed platelet aggregation inhibitory effects significantly different from controls. In general, the most active compounds inhibited both the primary (related to the receptor initial events) and secondary (irreversible, due to the release of agonists from the dense granules) phases of the in vitro aggregation. Compounds showing inhibitory effects significantly different from controls ($p < 0.001$) were subjected to a screening of dose-dependent effects on the in vitro aggregation of human PRP and concentrations inhibiting platelet aggregation by 50% (IC_{50}) were determined. The results are summarized in Table 1, where the % inhibition at 100 μM is reported as the mean (±SEM) of 3–4 individual determinations for weak inhibitors and the average IC_{50} values from 2–3 individual determinations for medium to strong inhibitors.

Table 2. Antiplatelet activity and lipophilicity of 4-hexyloxyphenyl esters and amides of piperidine carboxylic acids



Compd	Position on piperidine ring	Y	IC_{50} (μM) or % inhibition at 100 μM[a]		log P[b]	Half-lives in 10% human serum at 37 °C (min)[c]
			ADP	Adrenaline		
Control			15±1%	15±1%		
23a	2	NH	32±7%***	29±3%***	4.54	s
23b	2	O	nd	nd	4.78	3.6
18a	3	NH	45.1	66.0	4.25	s
18b	3	O	100	38.0±5%***	4.52	50
24a	4	NH	141	75.1	3.49	s
24b	4	O	120		3.84	50

[a]See footnote (a) in Table 1; nd, not determinable, because of strongly unreproducible results.
[b]See footnote (b) in Table 1.
[c]Data are means of three determinations (RSD less than 5%); s, stable, that is, no hydrolysis observed within 2 h of incubation.

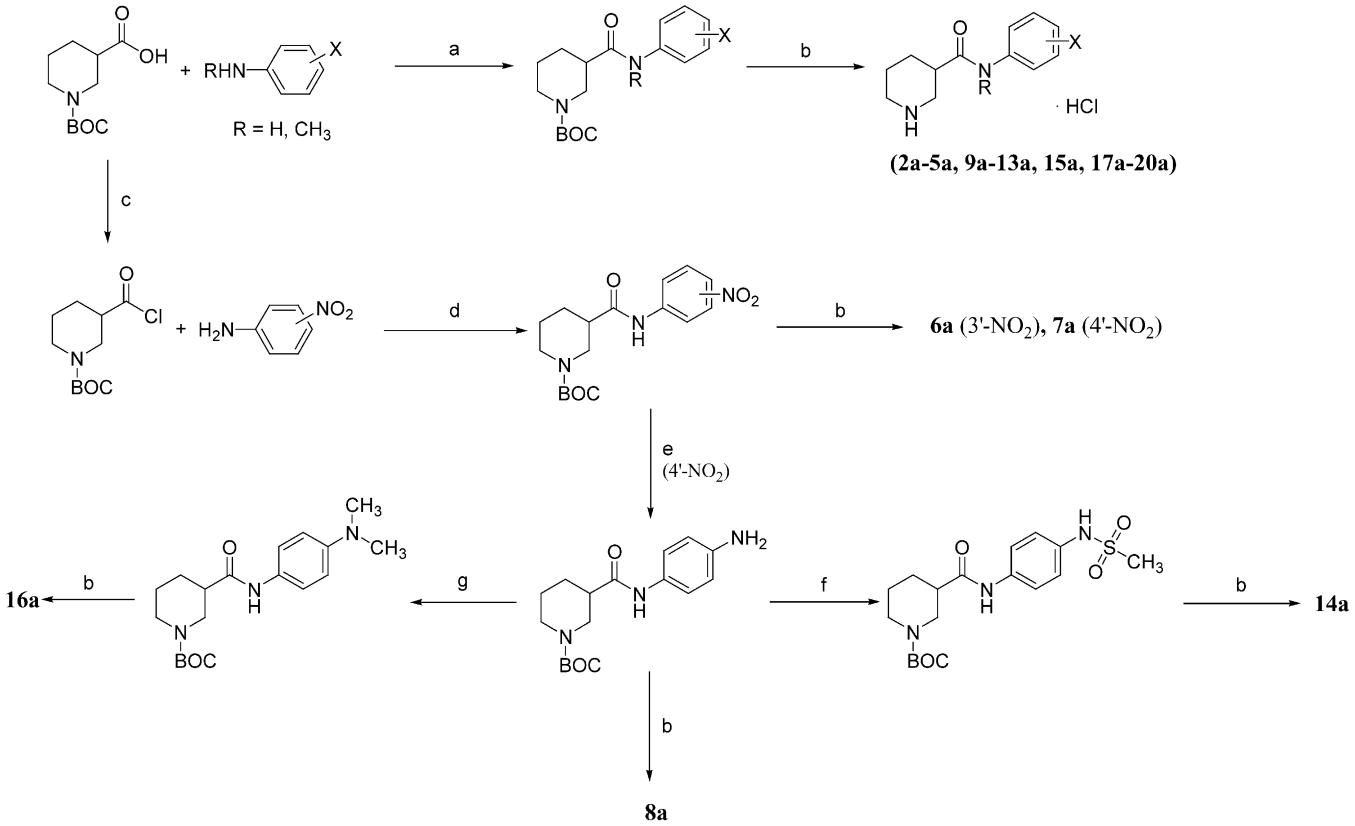
Among the compounds tested so far, 4-hexyloxyphenyl derivatives of nipecotic acid (**18a** and **18b**) exhibited the highest inhibition of platelet aggregation. Their dose–activity relationships are shown in Figure 2.

para-Hexyloxyanilide of nipecotic acid (**18a**) attained 50% inhibition activity of ADP-induced aggregation at a concentration (45 μM) close to the IC_{50} value for the bis-3-carbamoylpiperidine **1a** (39 μM) determined by Gollamudi et al.[17] Importantly, it showed a degree of inhibitory activity similar to that of aspirin, taken as the reference standard, in adrenaline-induced PRP aggregation.

Structure–activity relationships

Aggregation data of Table 1 showed that, with the exception of 4-*tert*-butyl derivatives (**17a** and **17b**), the amides were about two times more active than the corresponding esters, and derivatives bearing substituents at the *para* position of the phenyl ring were significantly more active than the *meta*-substituted ones. Indeed, the *meta*-substituted congeners, irrespective of their physicochemical properties, did not show any detectable activity at 100 μM, whereas the substituents at the position *para* of the phenyl ring enhance the activity mainly as a function of their lipophilicity, at least for compounds having a log P value higher than ca. 2 as calculated by the CLOG P software.[36] Only the finite antiplatelet activity data (IC_{50}) were used in a Hansch-type regression analysis aimed at deriving quantitative structure–activity relationships (QSARs); the IC_{50} value of **19a**, the only one obtained by extrapolation from the aggregation inhibition data, was also excluded from the regression analysis. An inspection of the plot of pIC_{50} values against log Ps (Fig. 3) suggested a trend of correlation between the inhibition of ADP-stimulating platelet aggregation and lipophilicity.

A detailed analysis of the subsets of piperidine-3-carboxylic acid derivatives revealed that the single parameter log P accounts for about 92% ($r^2 = 0.918$) of the variance of the activities of the phenyl esters; the correlation with log P alone was, however, poorer within the anilide subset ($r^2 = 0.465$), even after omitting from the regression the strongest outliers **2a** and **13a**, the two compounds having the lowest lipophilicity. In an effort to understand how, besides lipophilicity, other physicochemical properties can affect the antiplatelet activity, albeit the limited range of biological values (about 1 log unit), we carried out a Hansch-type multiple linear regression (MLR) with cross-validation on a matrix in which each compound was described by log P, calculated molar refractivity (CMR)[36] and molar volume (MV) as parameters of bulkiness, Hammett sigma (σ), resonance and inductive sigma constants (σ_R and σ_I) as the electronic parameters. MV and sigma constants were calculated with the ACDLab program.[37] Data analysis was carried out by using the Q-PARVUS package ver. 3.0.[38] With the set of piperidinecarboxylic acid derivatives having IC_{50} values (compounds **2a** and **13a** omitted from regression), and retaining only



Scheme 1. (a) BtOH, DCC, dry CH_2Cl_2, 35–60%; (b) HCl gas, $CHCl_3$, 0–5 °C, 35–50%; (c) $SOCl_2$, TEA, CH_2Cl_2, reflux; (d) TEA, CH_2Cl_2, 46%; (e) H_2, 10% Pd/C, EtOH, 98%; (f) methanesulfonyl chloride, dry pyridine, dry dioxane, reflux, 67%; (g) H_2, 40% H_2CO, 10% Pd/C, EtOH, 91%.

Table 3. Physicochemical and spectroscopic data of the newly synthesized compounds

Compd	Mp (°C)	Molecular formula	IR (cm^{-1})[a]	^1H NMR data (δ, ppm)[b]
3a	234–236	$C_{12}H_{15}N_2OBr \times HCl$	3290, 1663, 780	10.76 (s, 1H), 9.33 (s, 2H), 8.07 (d, $J=3$, 1H), 7.63 (m, 1H), 7.33 (m, 2H).
4a	251–254	$C_{12}H_{15}N_2OBr \times HCl$	3445, 1684, 820	10.46 (s,1H), 8.27 (s, 2H), 7.58 (d, $J=8.8$, 2H), 7.47 (d, $J=8.8$, 2H).
5a	228–230	$C_{12}H_{15}N_2OCl \times HCl$	3457, 1627, 832	10.33 (s, 1H), 9.27 (s, 2H), 7.76 (d, $J=9$, 2H), 7.41 (d, $J=9$, 2H).
6a	189–192	$C_{12}H_{15}N_3O_3 \times HCl$	3413, 1686, 1548, 1353, 739	11.20 (s, 1H), 9.43 (s, 2H), 8.79 (t, $J=3$, 1H), 8.07(m, $J=9$, 2H), 7.66 (t, $J=3$, 1H).
7a	266–267	$C_{12}H_{15}N_3O_3 \times HCl$	3210, 1695, 1558, 1328, 857	11.23 (s, 1H), 9.30 (s, 2H), 8.30 (d, $J=9$, 2H), 7.99 (d, $J=9$, 2H).
8a	260–261	$C_{12}H_{17}N_3O \times 2HCl \times H_2O$	3420, 1659, 1516, 823	10.44 (s, 1H), 9.84 (s, 5H), 7.65 (d, $J=8.5$, 2H), 7.22 (d, $J=8.5$, 2H).
9a	266–270	$C_{13}H_{15}N_2OF_3 \times HCl$	3248, 1692, 840	10.87 (s, 1H), 9.20 (s, 2H), 7.94 (d, $J=9$, 2H), 7.71 (d, $J=9$ Hz, 2H).
10a	266–269	$C_{13}H_{15}N_3O \times HCl$	3251, 2219, 1702, 837	10.65 (s, 1H), 8.82 (s, 2H), 7.77 (d, $J=8.7$, 2H), 7.65 (d, $J=8.7$, 2H).
11a	214–217	$C_{13}H_{18}N_2O \times HCl$	3267, 1686, 822	10.43 (s, 1H), 9.39 (s, 2H), 7.58 (d, $J=9$, 2H), 7.11 (d, $J=9$, 2H), 2.50 (s, 3H).
12a	202–204	$C_{13}H_{18}N_2O_2 \times HCl$	3254, 1659, 1215, 1039, 781	11.42 (s, 1H), 9.35 (s, 2H), 7.40 (d, $J=3$, 1H), 6.65 (m, 3H), 3.70 (s, 3H).
13a	204–207	$C_{13}H_{18}N_2O_2 \times HCl$	3449, 1684, 1237, 1028, 834	10.26 (s, 1H), 9.40 (s, 2H), 7.60 (d, $J=9$, 2H), 6.88 (d, $J=9$, 2H), 3.70 (s, 3H).
14a	282–284	$C_{13}H_{19}N_3O_3S \times HCl$	3422, 1687, 1342, 1161, 840	11.20 (s, 1H), 10.83 (s, 1H), 9.80 (s, 2H), 7.84 (d, $J=9$, 2H), 7.47 (d, $J=9$, 2H), 3.25 (s, 3H).
15a	272–274	$C_{14}H_{18}N_2O_2 \times HCl$	3285, 1699, 1663, 840	10.83 (s, 1H), 9.36 (s, 2H), 7.95 (d, $J=9$, 2H), 7.81 (d, $J=9$, 2H), 2.50 (s, 3H).
16a	233–236	$C_{14}H_{21}N_3O \times 2HCl \times H_2O$	3423, 1679, 840	10.01 (s, 1H), 9.22 (s, 3H), 7.31 (d, $J=9$, 2H), 7.12 (d, $J=9$, 2H), 3.05 (s, 6H).
17a	225–228	$C_{16}H_{24}N_2O \times HCl \times H_2O$	3420, 1690, 840	10.40 (s, 1H), 9.40 (s, 2H), 7.64 (d, $J=9$, 2H), 7.41 (d, $J=9$, 2H), 1.23 (s, 9H).
18a	177–180	$C_{18}H_{28}N_2O_2 \times HCl$	3276, 2777, 1653, 1237, 829	10.15 (s, 1H), 9.06 (s, 2H), 7.48 (d, $J=9$, 2H), 6.83 (d, $J=9$, 2H), 3.88 (t, $J=6$, 2H), 1.80–1.54 (m, 2H), 1.45–1.20 (m, 6H), 0.85 (t, $J=6$ Hz, 3H).
19a	131–135	$C_{19}H_{30}N_2O_2 \times C_4H_4O_4$	2922, 1654, 1514, 1249, 1125, 836	($CDCl_3$) 9.99 (s, 1H), 8.76 (s, 2H), 7.09 (d, $J=9$, 2H), 6.91 (d, $J=9$, 2H), 6.21 (s, 2H), 3.94 (t, $J=7$, 2H), 3.38–3.04 (m, 10H), 2.98–2.90 (m, 1H), 2.00–1.20 (m, 12H).
20a	166–170	$C_{19}H_{30}N_2O \times HCl$	3351, 1673, 841	10.21 (s, 1H), 9.11 (s, 1H), 8.90 (s, 1H), 7.50 (d, $J=8.5$, 2H), 7.11 (d, $J=8.5$, 2H), 2.87 (t, $J=7$, 2H), 1.85–1.50 (m, 2H), 1.40–1.03 (m, 8H), 0.87–0.83 (m, 3H).
23a	192–193	$C_{18}H_{28}N_2O_2 \times HCl \times H_2O$	3435, 1654, 1240, 1027, 831	10.55 (s, 1H), 9.15 (s, 1H), 8.90 (s, 1H), 7.50 (d, $J=9$, 2H), 6.89 (d, $J=9$, 2H), 3.90 (t, $J=6.5$, 2H), 3.31–3.21 (m, 1H), 3.00–2.92 (m, 1H), 2.22–2.18 (m, 1H), 1.83–1.27 (m, 14H), 0.88–0.83 (m, 3H)
23b	135–136	$C_{18}H_{27}NO_3 \times HCl \times H_2O$	3449, 1767, 1199, 827	9.58 (s, 2H), 7.10 (d, $J=9$, 2H), 6.97 (d, $J=9$, 2H), 4.29–4.27 (m, 1H), 3.94 (t, $J=6$, 2H), 3.38–3.26 (m, 1H), 2.97–2.93 (m, 1H), 2.24–2.20 (m, 1H), 1.80–1.61 (m, 7H), 1.39–1.28 (m, 6H), 0.50 (t, $J=7$, 3H).
24a	181–183	$C_{18}H_{28}N_2O_2 \times HCl \times 2H_2O$	3458, 1653, 1238, 831	10.55 (s, 1H), 9.20 (s, 1H), 8.86 (s, 1H), 7.47 (d, $J=9$, 2H), 6.84 (d, $J=9$, 2H), 3.88 (t, $J=6$, 2H), 3.37–3.23 (m, 1H), 3.00–2.82 (m, 1H), 2.30–2.18 (m, 1H), 1.94–1.61 (m, 14H), 0.85 (t, $J=7$, 3H).
24b	212–214	$C_{18}H_{27}NO_3 \times HCl \times H_2O$	3298, 1754, 1250, 1170, 841	9.58 (s, 2H), 7.10 (d, $J=9$, 2H), 6.97 (d, $J=9$, 2H), 4.30–4.28 (m, 1H), 3.94 (t, $J=6$, 2H), 3.38–3.26 (m, 1H), 2.96–2.92 (m, 1H), 2.24–2.20 (m, 1H), 1.80–1.61 (m, 7H), 1.38–1.28 (m, 6H), 0.88–0.85 (m, 3H).

[a]Only the most significant absorption bands have been reported.
[b]All spectra have been recorded in DMSO-d_6, if not differently indicated. Chemical shifts are expressed in δ values; coupling constants are expressed in Hz; exchange with D_2O was used to identify NH protons. Only value of NH, aromatic and signals of significant protons are reported in the case of nipecotic acid derivatives; the multiplets of the piperidine protons were found within the following δ ranges: 3.60–2.50 (m, 5H) and 2.80–1.30 (m, 4H) ppm.

QSARs with the cross-validated (leave-one-out procedure, *loo*) parameter $q^2 > 0.5$, the following two-variable equation was obtained, which explains about 75% of the variance in the biological data:

$$pIC_{50} = 0.17(\pm 0.05)\log P - 0.46(\pm 0.21)\sigma_R + 3.05(\pm 0.16)$$
$$n = 14,\ r^2 = 0.7390,\ q^2 = 0.6374,\ s = 0.1518,\ F = 15.57 \quad (1)$$

where n represents the number of data points, r^2 the squared correlation coefficient, q^2 the *loo* cross-validation coefficient (as a figure of the 'internal' predictive ability of the QSAR model), s the standard deviation of the regression equation, F the statistical significance of fit; 95% confidence intervals of the regression coefficients are given in parentheses.

In order to assess the relative contribution of each independent variable, we computed regression on the matrix of autoscaled data,[39] obtaining the following standardized coefficients:

$$(pIC_{50})' = 0.57(\log P)' - 0.41(\sigma_R)' \quad (2)$$

Eq 2 demonstrated that hydrophobic interactions, as accounted for by log P term, play a dominant role in modulating the inhibitory activity of ADP-induced PRP aggregation of phenyl esters and amides of nipecotic acid. Besides log P, a significant importance can be ascribed to the electronic properties of the substituents in the *para* position of the phenyl ring; the electron-donating groups, especially due to mesomeric effects (a statistically poorer equation was indeed obtained with σ instead of σ_R), increase the antiplatelet activity of nipecotoyl derivatives. Replacing log P with bulkiness parameters MR and MV resulted in equations with lower predictive abilities ($q^2 = 0.45$ and 0.35 for the equations with MR and MV, respectively).

It is worthy to point out that eq 1 holds fairly well only for derivatives bearing substituents in the *para* position of the phenyl ring and having log P values higher than a minimum (ca. 2). In fact, not only nipecotamides **2a** (X = H) and **13a** (X = 4-OCH_3) behaved as outliers, but based on eq 1 the IC_{50} value of a compound like **16a**, bearing the strong electron-donor group (σ_R of 4-$N(CH_3)_2$ is −0.88), should be around 200 μM, whereas instead it is a much weaker inhibitor, most likely because of its lower-than-minimum lipophilicity.

A dependence of the platelet aggregation inhibitory activity upon the hydrophobicity of nipecotoyl derivatives was somewhat expected based on the QSAR of bis-nipecotamides **1**.[15] A statistically significant parabolic relationship between activity and log P had been obtained by regression analysis carried out on a series of compounds **1** bearing different alkyl groups on the amide nitrogen, and the optimum log P value calculated around 4.5. Eq 1, which is based on a set of piperidinecarboxylic acid derivatives different from those of the Gollamudi data set (i.e., amides and esters instead of amides only, mono- instead of bis-amides, aryl instead of alkyl amide moieties), did not incorporate a quadratic log P term, even though we could not exclude that the correlation between pIC_{50} and log P is a non-linear one for our molecular data set too. In fact, once a log P value of ca. 4.2 has been reached with the 4-hexyloxy nipecotanilide **18a**, further increments of lipophilicity due, for example, to methylation of the amide NH (**19a**), more lipophilic substituent (**20a**), or replacement of amide with ester group (**18b**), resulted in a 2-fold decrease of activity against ADP-induced PRP aggregation.

The significant contribution of the resonance sigma constant in eq 1 could be explained in the light of arguments brought up in the literature.[17] It had been hypothesized that a key interaction causing stabilization of platelet membrane would be the binding of nipecotamides **1** to membrane phosphoinositides, and computational evidence had been reported that highlighted H bond formation between the 3-OH group of the inositol portion of phosphoinositides and the amide oxygen of nipecotamides as a HB acceptor. The strength of this HB should increase with increasing electronegativity of the carbonyl oxygen. Such an argument was used to explain the better activities of *N*,*N*-diethylamides ($CONEt_2$) over ethyl esters (COOEt) and methylketones (COMe).[17,40] Alkyloxy substituents, such as the hexyloxy group in our set, due to their mesomeric electron-donor properties, are those that render phenyl amides more similar to alkyl amides from the electronic property point of view, their electronic parameters of resonance being superimposable (σ_R are −0.15 and −0.14 for alkyloxyphenyl and alkyl groups, respectively). Within the series examined in this study, the importance of the electron-donor mesomeric effects is demonstrated by a number of pairwise comparisons. Thus, for example, 4-methyl anilide (**11a**), despite its lower lipophilicity, is 1.4-fold more active than the 4-trifluoromethyl congener (**9a**).

In summary, our data showed that, while the presence of a double amonium head should not be an essential requisite, optimal lipophilicity and electron-donor properties are major properties eliciting antiplatelet activity from piperidine-3-carboxamides. Based on a structural analogy criterion, it is conceivable that nipecotanilides may act through a mechanism similar to that of the bis-nipecotamides **1**. Therefore, using molecular modeling methods we examined the interaction of the most active derivative **18a** with phosphatidylinositol (PI), a major lipid component of the platelet membrane inner layer, taken as the main platelet target site.[41–43] Molecular models of the ligate PI and ligand **18a** (*R* and *S* configurations) were built using the SYBYL fragmental library,[44] and the aliphatic chains in both structures fixed in extended conformations. Flexible docking calculations were performed using the DYNDOCK algorithm of QXP software package,[45] using as starting geometries the minimum-energy conformations as found by molecular dynamics (AMBER/MM2 force field, 1000 cycles, 300 K, time

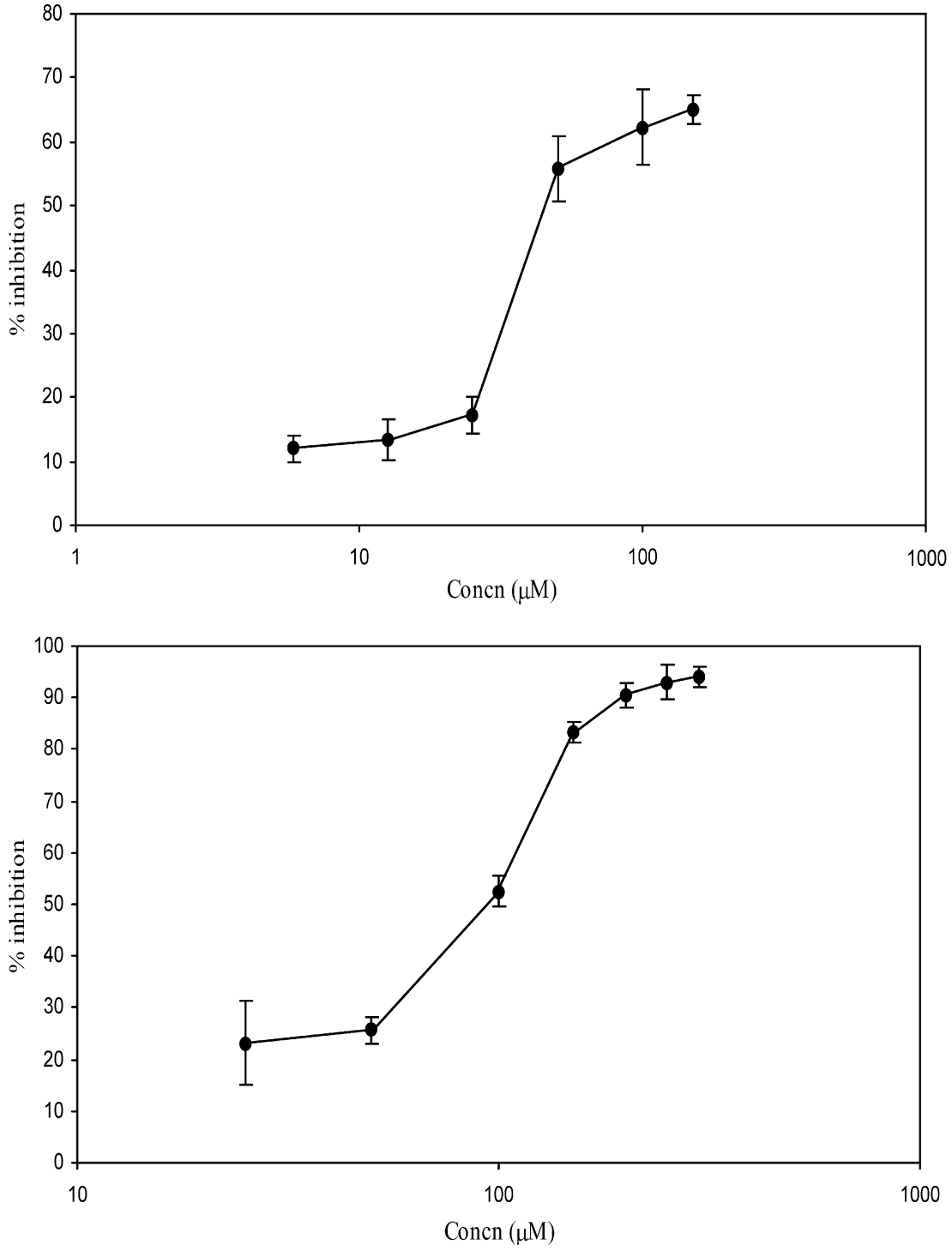


Figure 2. Concentration–response curves in logarithmic scale of compounds **18a** (top) and **18b** (bottom) on the platelet aggregation induced by ADP

step: 3 fs). Figure 4 shows the lowest-energy docked structures in the most populated cluster, and GRID contours[46] (calculated with water probe) highlight hydrophobic (yellow) and polar (blue) interactions. The calculated binding energy (−10.1 and −8.1 kcal/mol for *R*-**18a** and *S*-**18a**, respectively) indicated that nipecotamide **18a** (especially in *R*-configuration), similarly to bis-nipecotamide **1a**, may form a stable complex with PI.

In an additional QSAR study, we used the same independent variables of eq 1 in the regression analysis of another data set taken from literature,[17] that is bis-nipecotamides **1** with varying aromatic bridges connecting the two nipecotamide moieties (e.g., substituted-*p*-xylene connecting bridge, structure **1** in Fig. 1). The 'best' two-parameter equation and the corresponding standardized regression coefficients[39] obtained by the stepwise regression analysis are as follows:

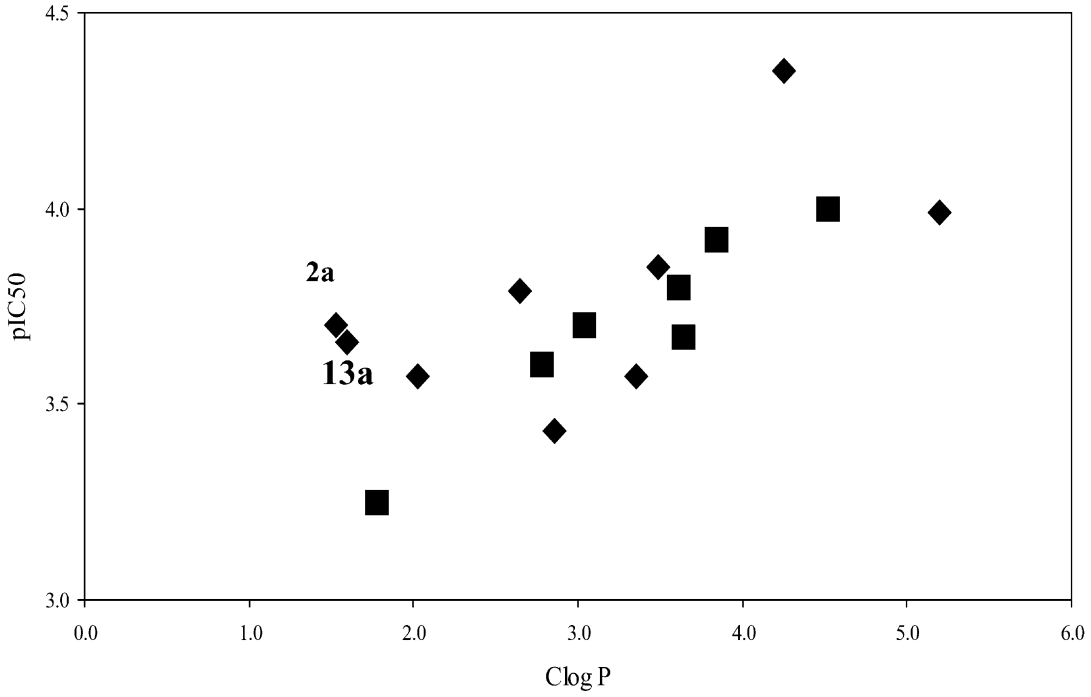


Figure 3. Plot of pIC_{50} against log P of piperidin-3-carboxylic acid derivatives (symbols: ◆ anilides, ■ esters).

$$pIC_{50} = 0.44(\pm 0.08)\log P - 0.58(\pm 0.26)\sigma + 2.12(\pm 0.32)$$
$$n = 15,\ r^2 = 0.8588,\ q^2 = 0.7969,\ s = 0.2940,\ F = 36.49 \quad (3)$$

$$(pIC_{50})' = 0.72(\log P)' - 0.30(\sigma)' \quad (4)$$

Eqs 3 and 4, which explain about 86% of the variance in the biological data, with a good predictive ability (q^2 of ca. 0.80), demonstrate that antiplatelet activity is primarily correlated with the lipophilicity of the connecting bridge, as graphically shown in Figure 5.

Moreover, the significant contribution of σ (slightly poorer statistics were obtained using σ_R in this case), that multiplies a negative coefficient, proved the increased activity to be also related to an increase in electron density of the aromatic bridge. The worst-fitting compound was the 2′-amino derivative **1b**; it was indeed omitted from the regression eq 3.

Eq 3 derived in our present study is much better, from a statistical viewpoint, than the QSAR reported by Gollamudi and coworkers in the original paper, which involved MV, MV^2 and sigma as the independent variables. We recomputed that regression equation, using the cross-validation procedure as a diagnosis of its prediction ability, and found a negative q^2 (i.e., no model at all) for the whole set of 16 compounds and q^2 of 0.576 after omitting compound **1a** from the data set, as did the authors, which is one of the most active derivatives within the series. In contrast, eq 3 showed statistics of some interest even when applied to the whole data set ($n = 16$, $r^2 = 0.715$, $q^2 = 0.548$).

Eqs 3 and 4, quite similar in their physicochemical meaning to eqs 1 and 2, showed that hydrophobicity and aromatic electron density have to be considered the main forces involved in the molecular recognition of nipecotamides at the platelet target site.

We finally wanted to compare activities of the nipecotoyl *p*-hexyloxyphenyl derivatives **18a** and **18b** with the isomeric pipecolinic (**23a** and **23b**) and isonipecotic (**24a** and **24b**) acid derivatives, and the results are summarized in Table 2.

Isonipecotamide **24a** was about 1/3 as active as its isomer nipecotamide **18a** in inhibiting the ADP-induced (and not the adrenaline-induced) PRP aggregation. Likely, once again lipophilicity may account for such a different antiplatelet properties, since compound **24a** is significantly less lipophilic than **18a**. In contrast, a difference in lipophilicity is highly unlikely to account alone for the observed difference in activity between nipecotamide **18a** and pipecolinamide **23a** (their lipophilicity differs in just 0.3 log P units).

In Table 2, the data of stability determined in buffered aqueous solution containing human serum could account for variations in activity of the phenyl esters. Nipecotate **18b** and isonipecotate **24b** were found to be stable enough during the time of the aggregation test (the hydrolyzed fraction was less than 15%), whereas pipecolinate **23b** underwent a fast cleavage in human serum solution at 37 °C, its half-life being less than 4 min. The fact that pipecolinic acid ester **23b**, similar to α-amino acid esters or related short-chained aliphatic amino acid esters,[47] is less resistant than the isomers **18b** and **24b** to the chemical (results not shown) and serum esterase-catalyzed hydrolysis can be a consequence of either the electron withdrawing effect of the protonated amino group, which activates the ester linkage toward OH^- attack, and (predominantly) the intramolecular

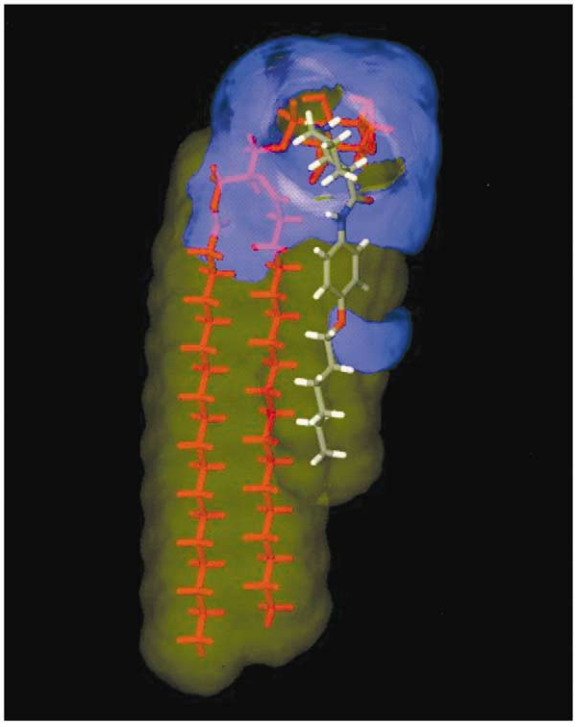


Figure 4. View of the complex *R*-**18a**/PI as resulted from flexible docking calculations performed with DYNDOCK algorithm of the QXP software.[45] The lowest-energy docked structures of the ligand, *R*-**18a**, and the ligate, PI, in the most populated cluster are represented (calculated binding energy: −10.1 kcal/mol). The molecular interaction fields are visualized as favorable H-bonding regions (blue-colored, contour level: −3.0 kcal/mol) and hydrophobic interaction regions (yellow-colored, contour level: +1.5 kcal/mol) derived from GRID calculations[46] with a water probe.

catalysis by the same neighboring protonated amino group that promotes ester cleavage. Due to its poor stability in plasma solution, pipecolinate **23b** did not show reproducible effects in the PRP aggregation assay.

Conclusions

A number of simple phenyl amides of piperidine-3-carboxylic acid (nipecotanilides), designed with the aim of simplifying the α,α′-bis[3-(*N*,*N*-dialkylcarbamoyl)piperidino]-*p*-xylenes (**1**) reported by others as potential antithrombotic agents,[15,17] exhibited appreciable and dose-dependent inhibition activities on platelet aggregation induced by ADP and adrenaline. Lipophilicity and presence of electron-donating groups in the *para* position of the phenyl ring were detected as the properties mainly responsible for the increase in activity. A log P value of 4.2 appeared desirable for optimum PRP aggregation inhibition within the examined property space, and *N*-[4-(hexyloxy)phenyl]piperidine-3-carboxamide hydrochloride (**18a**) was found as active as aspirin in inhibiting adrenaline-induced platelet aggregation. Docking calculations provided computational support to complex formation between the nipecotoyl anilide **18a** and phosphatidylinositol, a major component of the inner layer of the platelet membranes. This interaction may be considered as a key event triggering the antiplatelet action of nipecotamides.[24,25] Work is in progress to evaluate the effects of other modifications of the chemical structure of nipecotamides and to gain more information into their mechanism of action.

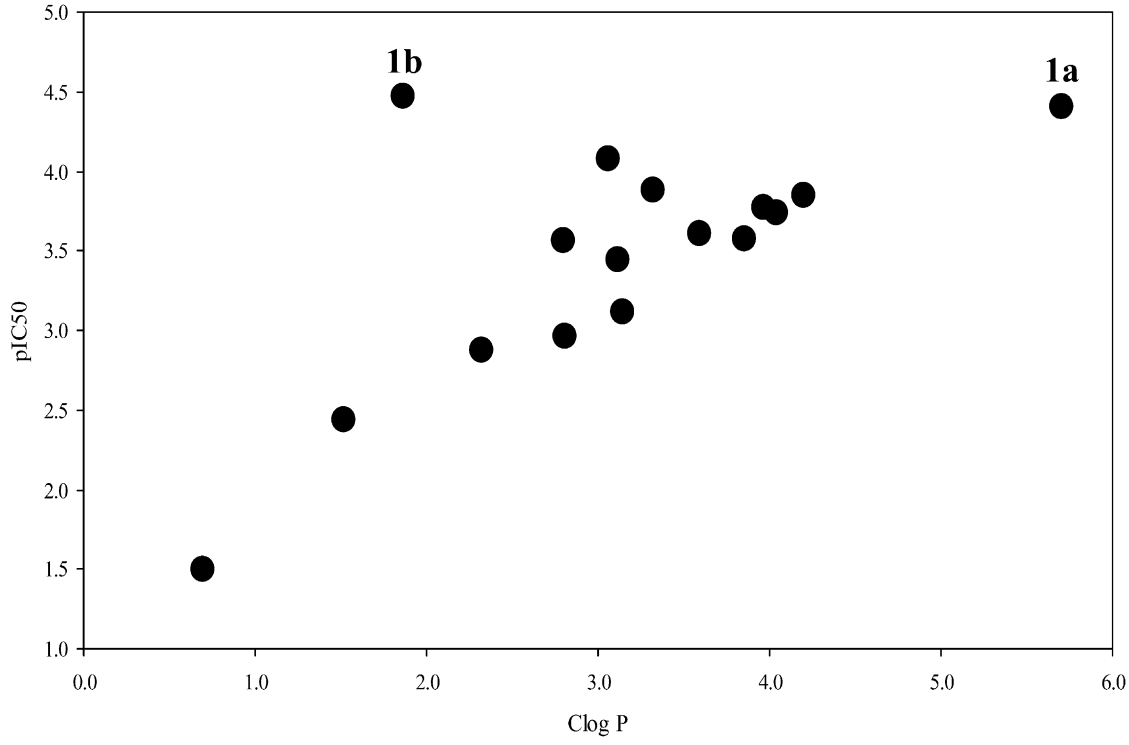


Figure 5. Plot of pIC_{50} against Clog P of α,α′-bis[3-(*N*,*N*-dialkylcarbamoyl)piperidino]-*p*-xylene analogues (Gollamudi data set).[17]

Experimental

Chemistry

Melting points were determined in open capillary tubes by SMP3 Stuart Scientific melting point apparatus and are uncorrected. Infrared absorption spectra were recorded as KBr pellets using a Perkin-Elmer Spectrum One FT IR instrument (Perkin-Elmer Ltd, Buckinghamshire, UK). ^{1}H NMR spectra were recorded on a Varian 300 MHz instrument. Chemical shifts are expressed in δ values and the coupling constants in Hz; exchange with D_2O was used to identify NH protons. The following abbreviations have been used: s, singlet; d, doublet; t, triplet; m, multiplet(s). Elemental analysis were performed by the Analytical Laboratory Service of the Dipartimento Farmaco-chimico of University of Bari on Euro EA3000 analyzer (Eurovector, Milan, Italy) and agreed with theoretical values to within ±0.40%. IR, ^{1}H NMR and elemental analysis data were consistent with the reported structures (Table 3). Chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI). Silica gel 60 (Merck 70–230 mesh) was used for column chromatography.

General procedure for the synthesis of *N*-phenylpiperidine carboxamides. As an example, the synthesis of *N*-phenylpiperidine-3-carboxamide hydrochloride (**2a**) is reported in detail. 1-(*t*-Butyloxycarbonyl)piperidine-3-carboxylic acid was prepared in 85% yield following reported method;[32] mp 148–150 °C (lit. Mp 149–151 °C).

N-BOC-nipecotic acid (1.0 g, 4.36 mmol), hydrated *N*-hydroxy-benzotriazole hydrated (0.6 g, 4.36 mmol) and dicyclohexylcarbodiimmide (0.9 g, 4.36 mmol) were suspended in 15 mL of dry dichloromethane. After stirring for 15 min, aniline (0.4 mL, 4.36 mmol) was added in one portion and the mixture stirred at rt for 10 h. Solid dicyclohexylurea (DCU) was filtered off, and, after removal of the solvent, the residue was dissolved in 50 mL of ethyl acetate and washed with 1 N HCl (3×20 mL), 5% $NaHCO_3$ (3×20 mL), and finally with water (3×20 mL). The organic phase was dried (Na_2SO_4) and evaporated under reduced pressure to yield generally a solid upon trituration with diethyl ether. Following purification of the solid residue by silica gel column chromatography (mobile phase: petroleum ether/ethyl acetate, 70:30 v/v), the product (0.80 g, 60% yield) was subjected to the subsequent reaction for the removal of the *N*-BOC-protecting group.

A solution of 1 mmol of the *N*-BOC-protected nipecotanilide in 100 mL of chloroform was cooled to 0–5 °C and saturated with HCl gas. The solvent was removed under reduced pressure to yield a solid. Recristallyzation from ethanol-ethyl acetate yielded the desired *N*-phenylpiperidinecarboxamide as hydrochloride salt. The total yields were in the range 30–40%.

***N*-(4-Nitrophenyl)piperidine-3-carboxamide hydrochloride (7a).** Thionyl chloride (0.70 mL, 8.8 mmol) was added dropwise to a cold stirred mixture of *N*-BOC-nipecotic-acid (1.0 g, 4.60 mmol) and triethylamine (1.2 mL, 8.80 mmol) in 40 mL of dichloromethane, and the mixture heated to 90 °C for 1 h. The solution was cooled to 0–5 °C and 4-nitro aniline (0.6 g, 4.36 mmol) in 30 mL of methylene chloride was added dropwise, then the reaction mixture was allowed to stir at rt overnight. Evaporation of the solvent under reduced pressure yielded a solid residue, which was purified by silica gel column chromatography (mobile phase: chloroform/methanol, 98:2 v/v) to give 0.70 g of the *N*-BOC-protected product (46% yield), which was subsequently deprotected to give, after crystallization from ethanol–ethyl acetate, 0.4 g of **7a** (32% yield).

Compound **6a** was synthesized using the same procedure.

***N*-(4-Aminophenyl)piperidine-3-carboxamide hydrochloride (8a).** A suspension of 0.5 g of **7a** (1.75 mmol) and 0.15 g of 10% Pd/C in 30 mL of ethanol was maintained for 8 h at rt under hydrogen atmosphere, until TLC analysis revealed the complete disappearance of the starting material. The reaction mixture was filtered on Celite pad and the catalyst was washed with ethanol. The filtrate was evaporated under reduced pressure to give 0.45 g (98% yield) of the *N*-BOC-protected derivative **8a**, which was then deprotected with HCl gas to give 0.21 g of **8a** (51% yield).

***N*-{4-[(Methylsulfonyl)amino]phenyl}piperidine-3-carboxamide hydrochloride (14a).** Methanesulfonylchloride (0.1 mL, 1.3 mmol) was added dropwise to a stirred solution of **8a** as *N*-BOC-derivative (0.3 g, 1.0 mmol) in 10 mL of dry dioxane and 1 mL of dry pyridine, upon cooling to 0–5 °C. The mixture was refluxed for 1 h, and then poured into ice-cold 2 N HCl. The resulting solution was extracted with chloroform (3×10 mL). The extracts were combined, dried (Na_2SO_4), and evaporated under reduced pressure to yield an oil, which solidified upon trituration with diethyl ether to give 0.25 g (67% yield) of *N*-BOC-protected product. Subsequent removal of the BOC-protecting group with HCl gas yielded 0.12 g of **14a** (38% yield).

***N*-[4-(Dimethylamino)phenyl]piperidine-3-carboxamide hydrochloride (16a).** A solution of the *N*-BOC derivative of **8a** (0.3 g, 1.0 mmol) and 40% aqueous formaldehyde (0.14 mL, 1.9 mmol) in 20 mL of ethanol was stirred at rt temperature under hydrogen atmosphere in the presence of 10% Pd/C (0.1 g) for 6 h. The reaction mixture was filtered on a Celite pad and the catalyst washed with ethanol. The combined filtrate, after concentration under reduced pressure, gave an oil which solidified upon trituration with diethyl ether. The solid residue was purified by silica gel column chromatography (mobile phase: petroleum ether/ethyl acetate, 70:30 v/v) to yield 0.3 g of the *N*-BOC-protected product, which gave 0.12 g of **16a** (38% yield) after the BOC-deprotection reaction with HCl gas.

***N*-[4-(Hexyloxy)phenyl]-*N*-methylamine.** A suspension of 4-hexyxloxy-aniline (1g, 5.2 mmol) in 20 mL of ethanol containing 40% aqueous formaldehyde (0.4 mL, 5.2

mmol) and 10% Pd/C (0.2 g) was stirred at rt under hydrogen atmosphere for 16 h. The reaction mixture was filtered on a Celite pad and the catalyst washed with ethanol. The combined filtrate was evaporated under reduced pressure to give an oil residue, which was purified by silica gel column chromatography (mobile phase: petroleum ether/ethyl acetate, 70:30 v/v) to afford 0.60 g of the product (56% yield).

***N*-(4-hexyloxyphenyl)-*N*-methylpiperidine-3-carboxamide maleate (19a).** *N*-BOC-protected nipecotic-acid (1.00 g, 4.36 mmol), hydrated *N*-hydroxy-benzotriazole (0.6 g, 4.36 mmol) and dicyclohexylcarbodiimmide (0.9 g, 4.36 mmol) were suspended in 20 mL of dry dichloromethane. After stirring for 15 min, *N*-methyl-4-hexyloxyaniline (0.9 g, 4.36 mmol) in 5 mL of dry dichloromethane was added dropwise. After stirring overnight at rt, the solid dicyclohexylurea was filtered off, and the solvent removed by evaporation under reduced pressure. The residue was dissolved in 50 mL of ethyl acetate and washed with 1N HCl (3×20 mL), 5% $NaHCO_3$ solution (3×20 mL), and finally with water (3×20 mL). The ethyl acetate solution was dried over Na_2SO_4, and evaporated under reduced pressure to yield an oil, which solidified upon trituration with diethyl ether. The solid residue was then purified by silica gel column chromatography (mobile phase: petroleum ether/ethyl acetate, 80:20 v/v) and the obtained product BOC-deprotected with HCl gas. The free base, obtained by treatment with $NaHCO_3$ and extraction with chloroform, was dissolved in ethyl acetate and added dropwise with an equimolar solution of maleic acid in isopropyl alcohol dropwise. Upon cooling **19a** was obtained as maleate salt (0.30 g, 16% yield).

Hydrolysis catalyzed by human serum

The susceptibility of the ester derivatives **18b**, **23b** and **24b**, and the corresponding amides toward hydrolysis catalyzed by human serum esterases was measured in phosphate buffer (40 mM, pH 7.4; ionic strength of 0.5 was maintained by adding a calculated amount of KCl) containing 10% of human serum at 37±0.2 °C. Each reaction were initiated by adding 100 μL of the methanolic stock solution of compound under examination to 1.9 mL of preheated serum solution (final concentration about 1×10^{-3} M) and the mixture was maintained in water bath at 37 °C. At appropriate times, 100 μL samples were withdrawn and added to 500 μL of cold acetonitrile in order to deproteinize the serum. After mixing and centrifugation (10 min at 4000 rpm), 5 μL of the clear supernatant were filtered through 0.2 μm membrane filter (Waters, PTFE 0.2 μm) and analyzed by HPLC. HPLC analysis was carried out on a Waters 1525 apparatus (constant-flow binary pump equipped with Waters 2487 variable wavelength detector). A Symmetry C_{18} column, 150×4.6 mm i.d. (Waters, Milan, Italy), was used as the stationary phase, and suitable water/MeOH mixtures were used as the mobile phases. A constant flow rate of 1.0 mL/min was maintained and the column effluent was continuously monitored at 254 nm. Quantification of the compounds was accomplished by measuring peak areas in relation to those of external standards.

For comparison, hydrolysis reactions were studied also in the absence of human serum. Pseudo-first-order rate constants for the hydrolysis were determined from the slopes of linear plots of the logarithm of the residual piperidine carboxylic ester against time.

Measurements of inhibitory activity of platelet aggregation

The effects on the in vitro aggregation of human platelet-rich plasma (PRP) induced by ADP and adrenaline were determined by the turbidimetric method of Born,[35] as described by Quintana et al.,[19,30] using a four-channel aggregometer PACKS-4 (Helena Laboratories, Beaumont, TX, USA).

All blood and blood products were handled in plastic ware, except for siliconized glass cuvettes and siliconized stir bars. With the sole exception of compound **19a** prepared as maleate salt, all the test compounds were prepared as hydrochlorides and dissolved in redistilled water containing dimethylsulfoxide (0.5% v/v final concentration). Solutions of the PRP aggregation inducers were prepared by dissolving ADP sodium salt or adrenaline in Tyrode's buffer (NaCl 137 mM, $NaHCO_3$ 11.9 mM, KCl 2.7 mM, NaH_2PO_4 0.36 mM, glucose 560 mM; pH 7.4 by dropwise addition of 1 N HCl). Human blood was obtained from healty volunteers who had not ingested any known antiplatelet drugs within three days prior to the blood drawing. Platelet-rich plasma (PRP) was obtained after centrifugation of venous blood (9 mL), mixed with 0.129 mol/L sodium citrate (1:9 to blood) to prevent it from clotting, at 100*g* for 10 min at 23 °C. PRP platelet numbers were counted and found as within the range 150,000–320,000/mL^{-1}.

PRP (250 μL) was pre-incubated with the test compound solutions (5 μL), or with dimethylsulfoxide (0.5% v/v, control) at 37 °C for 5 min, during which the suspension was stirred at 900 rpm. Then the inducer, 50 μL Tyrode's solution containing ADP (10 μM) or adrenaline (10 μM), was added to the stirred sample and the change in trasmittance at 640 nM was recorded for 10 min. The control cuvette containing the vehicle treated PRP followed the same sequence of events.

Inhibition of aggregation was expressed as a percent of the maximum response, and antiplatelet activity data were reported as means±SEM ($n=3$–6). A preliminary antiplatelet screen was obtained by measuring the effect of each compound at 100 μM concentration on ADP-induced aggregation. For compounds showing inhibitory activities significantly different from the respective control value, dose-response relations were determined in a concentration interval ranging from 12.5 to 400 μM and IC_{50} (concentration effecting 50% inhibition of aggregation) was obtained by regression of aggregation inhibition on log concentration of test compound. Aspirin was used a reference standard in the adrenaline-induced PRP aggregation assay.

Supplementary Table. Elemental analysis data of the newly synthesized compounds

Compd	Molecular formula	Elemental analysis (C, H, N,)					
		Calculated			Found		
		C	H	N	C	H	N
3a	$C_{12}H_{15}N_2OBr\times HCl$	45.09	5.05	8.76	45.30	5.03	8.83
4a	$C_{12}H_{15}N_2OBr\times HCl$	45.09	5.05	8.76	45.33	5.19	8.83
5a	$C_{12}H_{15}N_2OCl\times HCl$	52.36	5.86	10.18	52.44	5.98	10.32
6a	$C_{12}H_{15}N_3O_3\times HCl$	50.44	5.64	14.71	50.40	5.60	14.70
7a	$C_{12}H_{15}N_3O_3\times HCl$	50.44	5.64	14.71	50.66	5.82	14.71
8a	$C_{12}H_{17}N_3O\times 2HCl\times H_2O$	46.46	6.82	13.55	46.56	6.81	13.59
9a	$C_{13}H_{15}N_2OF_3\times HCl$	50.58	5.22	9.07	50.65	5.46	9.25
10a	$C_{13}H_{15}N_3O\times HCl$	58.76	6.07	15.81	59.01	6.25	15.88
11a	$C_{13}H_{18}N_2O\times HCl$	61.29	7.52	11.00	61.30	7.70	11.21
12a	$C_{13}H_{18}N_2O_2\times HCl$	57.67	7.07	10.35	57.68	7.01	10.38
13a	$C_{13}H_{18}N_2O_2\times HCl$	57.67	7.07	10.35	57.83	7.34	10.40
14a	$C_{13}H_{19}N_3O_3S\times HCl$	46.77	6.04	12.58	46.90	6.21	12.70
15a	$C_{14}H_{18}N_2O_2\times HCl$	59.47	6.77	9.91	60.09	7.02	10.02
16a	$C_{14}H_{21}N_3O\times 2HCl\times H_2O$	49.71	7.45	12.42	49.92	7.50	12.48
17a	$C_{16}H_{24}N_2O\times HCl\times H_2O$	61.04	8.64	8.90	61.23	8.64	8.82
18a	$C_{18}H_{28}N_2O_2\times HCl$	63.42	8.57	8.22	63.78	8.59	8.44
19a	$C_{19}H_{30}N_2O_2\times C_4H_4O_4$	63.57	7.89	6.45	63.67	8.33	6.43
20a	$C_{19}H_{30}N_2O\times HCl$	67.33	9.22	8.27	67.47	9.35	8.45
23a	$C_{18}H_{28}N_2O_2\times HCl\times H_2O$	60.24	8.71	7.80	60.47	8.77	8.09
23b	$C_{18}H_{27}NO_3\times HCl\times H_2O$	60.07	8.40	3.89	60.19	8.36	4.00
24a	$C_{18}H_{28}N_2O_2\times HCl\times 2H_2O$	57.36	8.82	7.43	57.60	8.86	7.56
24b	$C_{18}H_{27}NO_3\times HCl\times H_2O$	60.07	8.40	3.89	60.21	8.66	4.01

Acknowledgements

Part of this study was financially supported by Italian Ministry of Universities and Research (MIUR, Rome, Italy). The expert assistance of Dr. M. Micelli (Laboratorio di coagulazione, Policlinico Bari) in antiplatelet testing is gratefully acknowledged.

References and Notes

1. Jang, I.K.; Fuster, V. In *Thrombolitic Therapy*; Comerota, A. J. Ed.; J.B. Lippincott Co.: PA, 1995; p 17.
2. Majerus, W.; Broza, G.J.; Miletich, J.P.; Tollesfsen, D.M. In *The Goodman Gilman's Pharmacological Basis of Therapeutics*; Goodman, L. S., Gilman, A. G., Limbird, L. E. Eds.; McGraw-Hill: New York, 1996; p 1341.
3. Lam, J. Y. T.; Chesebro, J. H.; Steele, P. M.; Dewanjee, M. K.; Badimon, L.; Fuster, V. *J. Am. Coll. Cardiol.* **1986**, *8*, 1380.
4. Edmunds, L. H. *Ann. Thorac. Surg.* **1987**, *44*, 430.
5. Solymoss, B. C.; Nadeau, P.; Millette, D.; Campeau, L. *Circulation* **1988**, *78* (Suppl I), 140.
6. Fuster, V.; Pumphrey, C. W.; McGoon, M. D.; Chesebro, J. H.; Pluth, J. R.; McGoon, D. C. *Circulation* **1982**, *66* (Suppl I), 157.
7. Gachet, C. *Thromb. Haemost.* **2001**, *86*, 222.
8. Lages, B.; Weiss, H. J. *Thromb. Haemost.* **1980**, *43*, 147.
9. Kunapuli, S. P. *Trends Pharmacol. Sci.* **1998**, *19*, 391.
10. Van De Graaff, E.; Steinhubl, S. R. *Ann. Inter. Med.* **2000**, *32*, 561.
11. Calverley, D. C. *Clin. Geriatr. Med.* **2001**, *17*, 31.
12. Berger, P. B. *Curr. Cardiol. Rep.* **1999**, *1*, 192.
13. Hankey, G. J. *Cerebrovasc. Dis.* **2001**, *11* (Suppl 2), 11.
14. Schror, K. *Drugs* **1995**, *50*, 7.
15. Feng, Z.; Gollamudi, R.; Dillingham, E. O.; Bond, S. E.; Lyman, B. A.; Purcell, W. P.; Hill, R. J.; Korfmacher, W. A. *J. Med. Chem.* **1992**, *35*, 2952.
16. Lawrence, W. P.; Howell, R. D.; Gollamudi, R. *J. Pharm. Sci.* **1994**, *83*, 222.
17. Guo, Z.; Zheng, X.; Thompson, W.; Dugdale, M.; Gollamudi, R. *Bioorg. Med. Chem.* **2000**, *8*, 1041.
18. Lasslo, A.; Quintana, R. P.; Johnson, R. W.; Naylor, J. L.; Dugdale, M. *Biochim. Biophys. Acta* **1984**, *772*, 84.
19. Quintana, R. P.; Lasslo, A.; Dugdale, M.; Goodin, L. L. *Thromb. Res.* **1981**, *22*, 665.
20. Lasslo, A.; Dillingham, E. O.; Gollamudi, R.; Carter-Burks, G. *Biochim. Biophys. Acta* **1987**, *923*, 443.
21. Lasslo, A.; Dillingham, E. O.; Gollamudi, R.; Carter-Burks, G. *Res. Commun. Chem. Pathol. Phrmacol.* **1987**, *58*, 129.
22. Quintana, R. P.; Lasslo, A.; Greer, T. *Thromb. Res.* **1981**, *24*, 379.
23. Bevers, E.M.; Rosing, J.; Zwaal, R.F.A. In *Platelets in Biology and Pathology—III*; MacIntyre, D.E., Gordon, J.L. Eds.; Elsevier: Amsterdam, 1987; p 135.
24. (a) Gollamudi, R.; Dillingham, E. O.; Bond, S. E. *Thromb. Haemost.* **1993**, *69*, 1322. (b) Lasslo, A.; Quintana, R. P.; Crisan, D.; Baier, R. E.; Mayer, A. E.; Fornalik, M. S. *Med. Prog. Technol.* **1984**, *10*, 71.
25. Lasslo, A. *Fed. Proc.* **1984**, *43*, 1382.
26. Gollamudi, R.; Dillingham, E. O.; Bond, S. E. *Blood. Coag. Fibrinol.* **1994**, *5*, 375.
27. Handa, R. K.; Dillingham, E. O.; Gollamudi, R. *Life Sci.* **1995**, *57*, 983.
28. Zheng, X.; Salgia, S. R.; Thompson, W. B.; Dillingham, E. O.; Bond, S. E.; Feng, Z.; Prasad, K. M.; Gollamudi, R. *J. Med. Chem.* **1995**, *38*, 180.
29. Dillingham, E. O.; Lasslo, A.; Carter-Burks, G.; Bond, S. E.; Gollamudi, R. *Biochim. Biophys. Acta* **1989**, *990*, 128.
30. Altomare, C.; Summo, L.; Varlamov, A. V.; Voskressensky, L. G.; Borisova, T. N.; Carotti, A. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 581.
31. Voskressensky, L.G.; de Candia, M.; Carotti, A.; Borisova, T.N.; Kulikova, L.N.; Valamov, A.V.; Altomare, C. *J. Pharm. Pharmacol.* in press.
32. Bonina, F. P.; Arenare, L.; Palagiano, F.; Saija, A.; Nava, F.; Trombetta, D.; De Caprariis, P. *J. Pharm. Sci.* **1999**, *88*, 561.
33. Altomare, C.; Carotti, A.; Cellamare, S.; Ferappi, M.; Cagiano, R.; Renna, G. *Int. J. Pharm.* **1988**, *48*, 91.
34. Hellberg, M.; Stubbins, J. F.; Glennon, R. A. *Bioorg. Med. Chem.* **2000**, *8*, 1917.
35. Born, G. V. R. *Nature* **1962**, *194*, 927.
36. *CLOG P for Windows*, vers. 4.0; BioByte Corp.: Claremont, CA, USA.
37. *ACD/Labs package*, release 5.0, Advanced Chemistry Development Inc.: Toronto, Ontario, Canada.
38. Forina, M.; Lanteri, S.; Armanino, C. *Q-PARVUS, vers. 3.0, An Extendable Package of Programs for Data Explorative Analysis, Classification and Regression Analysis*; Dipartimento di Chimica e Tecnologie Farmaceutiche ed Alimentari: Genova, Italy.
39. Mager, H.; Barth, A. *Pharmazie* **1979**, *34*, 557.
40. Quintana, R. P.; Lasslo, A.; Dugdale, M. *Biophys. J.* **1982**, *37*, 130.
41. Shattil, S. J.; Cooper, R. A. *Biochemistry* **1976**, *15*, 4832.
42. Chap, H.; Perret, B.; Manco, G.; Simon, M. F.; Douste-Blazy, L. *Agents Actions* **1979**, *9*, 400.
43. Nozawa, Y.; Nakashima, S.; Nogata, K. *Biochim. Biophys. Acta* **1991**, *1082*, 219.
44. *SYBYL software*, vers. 6.7; Tripos Associates: Saint Louis, MO, USA.
45. Mc Martin, C.; Bohacek, R. S. *J. Computer Aided Mol. Des.* **1997**, *11*, 333.
46. Goodford, P. J. *J. Med. Chem.* **1985**, *28*, 849.
47. Bundgaard, H.; Larsen, C.; Thorbek, P. *Int. J. Pharm.* **1984**, *18*, 67.