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Lipophilicity-related inhibition of blood platelet aggregation by nipecotic acid anilides

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Abstract

Using *N*-[4-(hexyloxy)phenyl]piperidine-3-carboxamide (**17c**) as a structural lead, a number of isomers, derivatives, and ring-opened analogs were synthesized and tested for their ability to block the in vitro aggregation of human platelets induced by adenosine 5′-diphosphate (ADP). For the most active compounds, inhibition of the platelet aggregation triggered by arachidonic acid (AA) and ADP-induced intraplatelet calcium mobilization was also demonstrated. Based on quantitative structure–activity relationships (QSARs), we proved the impact of hydrophobicity on antiplatelet activity by a nonlinear (parabolic or bilinear) relationship between pIC_{50} and lipophilicity, as assessed by RP-HPLC capacity factors and Clog *P* (i.e. calculated 1-octanol–water partition coefficients). This study highlighted the following additional SARs: quasi-isolipophilic isomers of **17c** (isonipecotanilides and pipecolinanilides) and ring-opened analogs (e.g. anilide of β-alanine) exhibited lower antiplatelet activity; methylation of the piperidine nitrogen of **17c** has no effect, whereas alkylation with an *n*-propyl group decreases the activity by a factor of approximately 2, most likely due to a conformation-dependent decrease in lipophilicity.

Keywords: Anilides of nipecotic acid; Platelet active drugs; Lipophilicity; Structure–activity relationships

1. Introduction

Platelets are involved in the pathogenesis of many cardiovascular and thromboembolic diseases (Fuster et al., 1992; Majerus and Tollesfsen, 2001). Their hyperactivity increases the risk of various vaso-occlusive diseases, such as unstable angina, acute myocardial infarction, transient ischemic attacks, and complications following percutaneous coronary intervention (Weksler, 2000).

Platelets can be activated by a number of agonists, such as adenosine 5′-diphosphate (ADP), thrombin, platelet activating factor (PAF), serotonin, thromboxane A_2 (TxA_2), collagen, and cathecolamines (Brass, 1995). ADP plays a relevant role in platelet function. It can trigger platelet activation, which is mediated by at least three purinergic receptors (P_2Y_1, P_2Y_{12}, P_2X) showing distinct specificity; ADP is also responsible for the secondary wave of platelet aggregation, followed by ADP release from dense granules which potentiates the aggregation response induced by other agents (Mills, 1996; Kunapuli, 1998; Gachet, 2001).

Antiplatelet agents, including aspirin and thromboxane modulators (e.g. ridogrel), ADP antagonists, like the thienopyridines ticlopidine and clopidogrel, phosphodiesterase inhibitors (e.g. dipyridamole and cilostazol), and platelet glycoprotein IIb/IIIa antagonists, such as tirofiban and sibrafiban, are useful in the prophylaxis and treatment of thromboembolic diseases (Van De Graaff and Steinhubl, 2000; Patrono et al., 2001; Dogné et al., 2001; Antithrombotic Trialists' Collaboration, 2002). Nevertheless, currently available, orally administered antiplatelet drugs have limitations, especially with regard to side effects and broad clinical utility, as well as interference with physiological platelet function in hemostasis (Patrono et al., 2001). For instance, ticlopidine and clopidogrel (Fig. 1), which act in vivo, via thiol metabolites (Ha-Duong et al., 2001; Dogné et al., 2001), on the platelet ADP receptor subtype P_2Y_{12}

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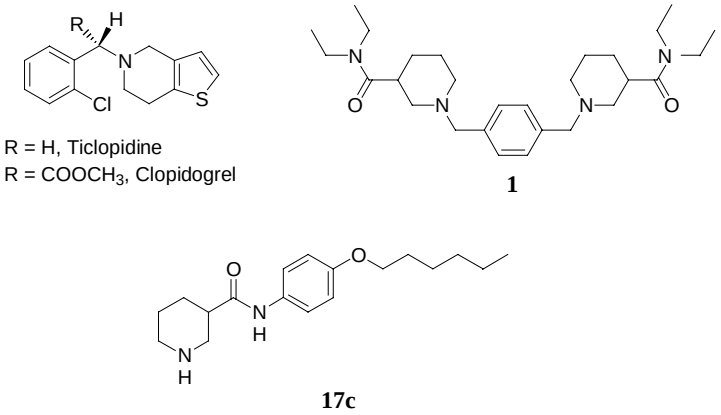


Fig. 1. Structures of antithrombotic agents ticlopidine and clopidogrel (in the *S*-configuration), general structure of the literature bis-3-carbamoylpiperidines **1**, and 4-hexyloxyanilide of nipecotic acid **17c** previously found to be a promising antiplatelet agent (de Candia et al., 2003).

(Hollopeter et al., 2001; Storey, 2001), have been increasingly used in order to prevent ischemic events, and their combined use with aspirin has been shown to be effective in suppressing thrombotic complications after coronary stenting and in other conditions with high risk of atherothrombotic events (Moussa et al., 1999; Mishkel et al., 1999; Hankey, 2001). Despite their efficacy, the administration of thienopyridines is associated with some undesired side effects, such as neutropenia, thrombocytopenia, aplastic anemia, and thrombotic thrombocytopenic purpura (Berger, 1999). Moreover, most probably due to selective inhibition of some pathways of platelet activation and recruitment by aspirin and thienopyridines, a number of patients have been shown to be resistant to these drugs (McKee et al., 2002; Gurbel et al., 2003). These limitations are among the reasons stimulating the search for new antiplatelet drugs.

Our interest in antiplatelet agents stemmed from the finding that some bis-3-carbamoylpiperidine derivatives (structure **1** in Fig. 1), namely α,α′-bis[3-(*N*,*N*-diethylcarbamoyl) piperidino]-*p*-xylenes, inhibit in vitro human platelet aggregation, and are effective in thrombosis models in vivo (Feng et al., 1992; Lawrence et al., 1994; Guo et al., 2000).

Due to their lipophilicity and surface activity, type **1** bis-nipecotamides can penetrate the platelet membranes and interact with anionic phospholipids (mainly phosphatidylinositol, PI, and phosphatidylserine, PS), thus 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). They inhibit 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 (Dillingham et al., 1989; Feng et al., 1992; Guo et al., 2000).

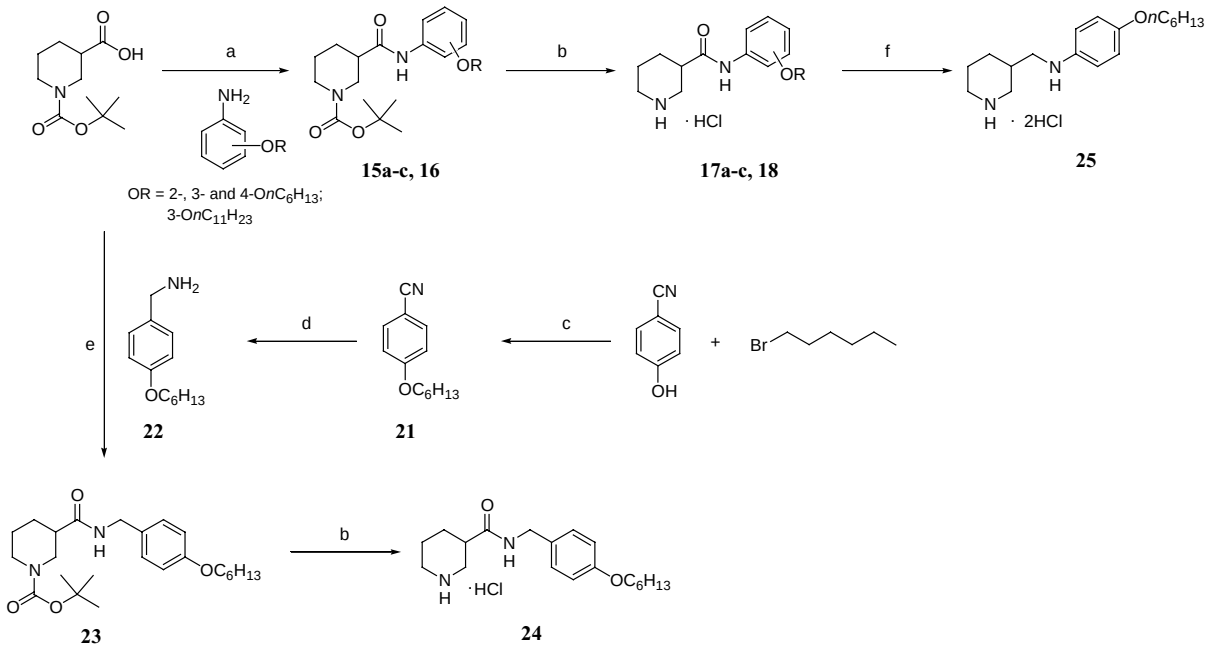
With the aim of gaining insights into the minimal structural features necessary for antiplatelet activity of nipecotamides, we recently undertook an investigation into a series of simple phenyl amides and esters of piperidin-3-carboxylic acid (nipecotic acid) bearing various substituents in the meta and para positions of the phenyl group (de Candia et al., 2003). As a general trend, the carboxamides were found to be more active than the corresponding esters, and para-substituted derivatives more active than the meta-substituted ones. 4-Hexyloxyanilide of nipecotic acid (compound **17c** in this paper; Fig. 1 and Table 2) proved to be a promising compound in vitro, its IC_{50} value being close to that of the most active bis-3-carbamoylpiperidines (Feng et al., 1992; Guo et al., 2000). Compared with the isomeric 4-hexyloxyanilides of piperidine-2-carboxylic (pipecolinic) and piperidine-4-carboxylic (isonipecotic) acids, compound **17c** showed higher activity, and a quantitative structure–activity relationship (QSAR) study highlighted hydrophobicity and electron-donor capacity of the para-substituents as the properties mainly related to antiplatelet activity (de Candia et al., 2003).

With the aim of establishing more detailed SARs in this series and possibly improving the activity, we synthesized and evaluated other lipophilic piperidine-3-carboxamides, most of them bearing the *n*-hexyloxy substituent on the phenyl ring. Herein, we report on the effects on the platelet aggregation inhibitory activity of the alkylation of the piperidine nitrogen and positional isomerism of lipophilic alkoxy substituents, the importance of the 3-amide group, the influence of the piperidine ring opening, and the impact of hydrophobicity on the activity. The effect of the most active compound on calcium mobilization was also evaluated.

2. Materials and methods

2.1. 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.



Scheme 1. (a) HOBt, DCC, dry CH_2Cl_2 (b) $CHCl_3$, HCl gas, 0 °C (c) NaH, dry DMF (d) H_2, 10% Pd/C, EtOH (e) HOBt, DIEA, DCC, dry CH_2Cl_2 (f) borane–trimethylamine complex, dioxane, reflux.

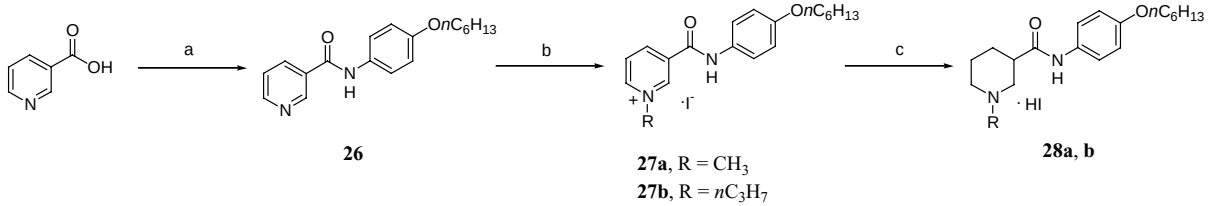
Mass spectra were registered on Agilent GC-MS 6890-5973. All newly synthesized compounds showed IR, ^{1}H NMR and mass spectra according with reported structures. Elemental analyses were performed using the Analytical Laboratory Service of the Dipartimento Farmaco-chimico of University of Bari on Euro EA3000 analyzer (Eurovector, Milan, Italy) and agreed within ±0.40% of the calculated values. Chemicals were purchased from Aldrich Chemical Company (Milwaukee, WI). Silica gel 60 was used for column chromatography (Merck 70–230 mesh, alternatively 15–40 mesh for flash chromatography). The newly synthesized alkoxyphenyl carboxamide derivatives were prepared according to Schemes 1 and 2. Product yields (not optimized), physical and spectral data of the newly synthesized compounds are reported in Table 1.

2.1.1. General procedure for the synthesis of alkoxyanilines

To a suspension of aminophenol (1.0 g, 9.2 mmol) and NaH (0.63 g, 27.5 mmol) in 10 ml of dry DMF, the selected 1-alkylbromide (13.7 mmol) was added and the mixture stirred at room temperature for 24 h. The reaction was quenched by adding 40 ml of distilled water and the aqueous layer was extracted with 3 × 20 ml of ethyl acetate. The collected organic phases were dried (Na_2SO_4) and concentrated to afford the corresponding alkoxyanilines in 80–90% yields. For each alkoxyaniline derivative, the spectroscopic data were consistent with structure and literature (3-hexyloxyaniline, Higuchi et al., 1997; 3-undecanyloxyaniline, Booth et al., 1987).

*2.1.2. Synthesis of 4-hexyloxy benzylamine (**22**)*

To obtain the intermediate 4-hexyloxybenzonitrile (**21**), a solution of 4-hydroxybenzonitrile (1 g, 8.4 mmol) in 10 ml of dry DMF was treated with NaH (0.51 g, 25.2 mmol). After few minutes, 1-bromohexane (2.1 g, 12.6 mmol) in 5 ml of dry DMF was added dropwise and the mixture stirred at room temperature till TLC revealed disappearance of the starting material (45 h). The reaction was quenched by adding 50 ml of distilled water and the aqueous phase extracted with 3 × 20 ml of ethyl acetate. The combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (mobile phase: petroleum ether/ethyl acetate, 70:30, v/v) to give 0.97 g (56%) of crystals (lit. m.p. 32 °C).



Scheme 2. (a) (1) $SOCl_2$, pyridine, toluene; (2) 4-hexyloxyaniline; (b) Alkyl (methyl, 1-propyl) iodide, reflux; (c) H_2, PtO_2, 60 psi, absolute ethanol.

Table 1
Physicochemical and spectroscopic data of the newly synthesized compounds

Compound	Yield[a] (%)	Mp (°C)	Molecular formula[b]	IR (cm^{-1})[c]	^1H NMR data (δ, ppm)[d]
17a	20	137–139	$C_{18}H_{29}N_2O_2Cl$	3340, 2927, 1691, 1534, 1451, 1214	0.83 (t, $J = 7$, 3H), 1.13–1.50 (m, 9H), 1.55–3.30 (m, 8H), 3.98 (t, $J = 7$, 2H), 6.83 (m, 1H), 6.99–7.40 (m, 2H), 7.62 (m, 1H), 8.91 (s, 1H), 9.05 (s, 1H), 9.15 (s, 1H)
17b	40	151–153	$C_{18}H_{29}N_2O_2Cl$	3346, 2924, 1685, 1595, 1449, 1215	0.92 (t, $J = 7$, 3H), 1.13–1.15 (m, 9H), 1.55–3.30 (m, 8H), 3.94 (t, $J = 7$, 2H), 6.60 (d, $J = 9$, 1H), 7.01–7.10 (m, 2H), 7.14 (s, 1H), 8.58 (s, 2H), 10.08 (s, 1H)
18	35	153–155	$C_{23}H_{39}N_2O_2Cl$	3356, 2922, 2851, 1685, 1595, 1450, 1216	0.83 (t, $J = 7$, 3H), 1.40–1.90 (m, 17H), 1.55–3.30 (m, 10H), 3.94 (t, $J = 7$, 2H), 6.60 (d, $J = 9$, 1H), 7.01–7.10 (m, 2H), 7.15 (s, 1H), 8.80 (s, 1H), 9.01 (s, 1H), 10.12 (s, 1H)
24	46	Hygroscopic solid		3419, 2933, 1647, 1513, 1247	0.93 (t, $J = 7$, 3H), 1.10-1.42 (m, 6H), 1.50–3.30 (m, 11H), 3.94 (t, $J = 7$, 2H), 4.08 (t, $J = 4$, 2H), 6.83 (d, $J = 9$, 2H), 7.07 (d, $J = 9$, 2H), 8.58 (t, $J = 4$, 1H), 8.85 (s, 1H), 9.03 (s, 1H)
25	43	Brown oil		3379, 2934, 2858, 1514, 1233, 818	($CDCl_3$) 0.80–2.00 (m, 20H), 2.95–3.20 (m, 4H), 3.88 (t, $J = 6.6$, 2H), 6.53 (d, $J = 9$, 2H), 6.78 (d, $J = 9$, 2H)
26	93	126–127	$C_{18}H_{22}N_2O_2$	3339, 2936, 1648, 1527, 1515, 1255, 824	($CDCl_3$) 0.83–0.88 (m, 3H), 1.28–1.41 (m, 6H), 1.65–1.70 (m, 2H), 3.92 (t, $J = 6.5$, 2H), 6.90 (d, $J = 9$, 2H), 7.53–7.56 (m, 1H), 7.63 (d, $J = 9$, 2H), 8.25 (d, $J = 8$, 1H), 8.72 (d, $J = 5$, 1H), 9.07 (d, $J = 2$, 1H), 10.29 (s, 1H)
27a	94	182–187	$C_{19}H_{25}N_2O_2I$	3300, 1660, 1513, 1238, 1029, 826	1.10–2.30 (m, 11H), 4.26 (t, $J = 6$, 2H), 4.76 (s, 3H), 7.28 (d, J = 9, 2H), 7.99 (d, $J = 9$, 2H), 8.36 (t, $J = 6$, 1H), 9.20–9.65 (m, 2H), 9.86 (d, $J = 3$, 1H), 10.9 (s, 1H)
27b	70	215–217	$C_{21}H_{29}N_2O_2I$	3443, 1665, 1512, 1252, 828	0.80–0.95 (m, 6H), 1.20–1.50 (m, 6H), 1.69 (qt, $J = 6.5$, 2H), 1.98 (q, $J = 7.5$, 2H), 3.94 (t, $J = 6.5$, 2H), 4.64 (t, $J = 7.5$, 2H), 6.96 (d, $J = 9$, 2H), 7.64 (d, $J = 9$, 2H), 8.30 (t, $J = 7$, 1H), 9.03 (d, $J = 8.3$, 1H), 9.22 (d, $J = 6$, 1H), 9.58 (s, 1H), 10.72 (s, 1H)
28a	63	158–159	$C_{19}H_{31}N_2O_2I$	3449, 2933, 1666, 1541, 1245, 831	0.87 (t, $J = 7$, 3H), 1.20–1.55 (m, 7H), 1.60–1.75 (m, 4H), 1.85–2.00 (m, 2H), 2.70–2.95 (m, 4H), 3.00–3.15 (m, 1H), 3.35–3.45 (m, 1H), 3.45–3.60 (m, 1H), 3.88 (t, $J = 6$, 2H), 6.85 (d, $J = 9$, 2H), 7.44 (d, $J = 9$, 2H), 9.24 (s, 1H), 9.99 (s, 1H)
28b	53	131–133	$C_{21}H_{35}N_2O_2Cl$	3425, 3049, 2934, 1667, 1511, 1236	0.80–1.00 (m, 6H), 1.20–2.10 (m, 14H), 2.78–3.10 (m, 5H), 3.36–3.50 (m 2H), 3.88 (t, $J = 6.6$, 2H), 6.84 (d, $J = 9$, 2H), 7.47 (d, $J = 9$, 2H), 10.34 (s, 1H), 10.75 (s, 1H)
29	30	228–229	$C_{15}H_{25}N_2O_2Cl$	3299, 2937, 1655, 1238, 822	0.85 (m, 3H), 1.27–1.37 (m, 6H), 1.63–1.68 (m, 2H), 3.03 (t, $J = 7$, 2H), 3.12 (t, $J = 7$, 2H), 3.80 (t, $J = 6$, 2H), 6.84 (d, $J = 9$, 2H), 7.48 (d, $J = 9$, 2H), 8.69 (s, 3H), 10.17 (s, 1H)
30	30	155–159	$C_{16}H_{27}N_2O_2Cl$	3413, 3312, 2937, 1655, 1531, 1240, 822	0.85 (m, 3H), 1.27–1.37 (m, 6H), 1.37–1.65 (m, 2H), 1.82–1.85 (m, 2H), 2.37 (t, $J = 7$, 2H), 2.80 (t, $J = 7$, 2H), 3.88 (t, $J = 6$, 2H), 6.83 (d, $J = 9$, 2H), 7.47 (d, $J = 9$, 2H), 7.90 (s, 3H), 9.93 (s, 1H)
31	29	181–185	$C_{17}H_{29}N_2O_2Cl$	3240, 2923, 1641, 1538, 1510, 1247, 818	0.75–1.80 (m, 11H), 2.00–2.30 (m, 6H), 2.72–2.76 (m, 2H), 3.89 (t, $J = 7$, 2H), 6.84 (d, $J = 9$, 2H), 7.58 (d, $J = 9$, 2H), 8.33 (s, 3H), 10.03 (s, 1H)

[a] Yields refer to purified products and were not optimized.
[b] In agreement with elemental analyses (C, H, N) within ±0.4% of the theoretical values.
[c] Only the most significant absorption bands have been reported.
[d] All spectra were recorded in d_6-DMSO, if not differently indicated. Chemical shifts are expressed in δ values; coupling constants (J) are expressed in hertz. Abbreviations: s, singlet; d, doublet; t, triplet; qt, quintet; m, multiplet(s). Exchange with D_2O was used to identify NH protons.

A suspension of 4-hexyloxybenzonitrile (1.0 g, 4.96 mmol) and a catalytic amount of 10% Pd/C in 20 ml of ethanol was stirred for 2 h under hydrogen atmosphere and room temperature and pressure. The suspension was filtered-off using a Celite pad and the filtrate concentrated under reduced pressure, affording 0.45 g oil (67%) of 4-hexyloxybenzylamine. MS: *m*/*z* 207 (M^+), 122 (base).

*2.1.3. Synthesis of substituted N-(alkoxyphenyl)alkylamino carboxamides hydrochlorides (**17a–c**, **18**, **24**, **29–31**)*

The synthesis of *N*-(3-hexyloxyphenyl)piperidine-3-carboxamide hydrochloride (**17b**) is reported as an example of the general procedure utilized.

A mixture of *N*-BOC–nipecotic acid (1.0 g, 4.4 mmol), hydrated *N*-hydroxy-benzotriazole (HOBt, 0.6 g, 4.4 mmol), dicyclohexylcarbodiimmide (DCC, 0.9 g, 4.4 mmol), ethyldiisopropylamine (DIEA, 0.76 ml, 4.4 mmol) and 3-hexyloxyaniline (0.84 g, 4.4 mmol) were suspended in 15 ml of dry dichloromethane and stirred overnight at room temperature. Solid dicyclohexylurea (DCU) was filtered-off and, after removal of the solvent under reduced pressure, the residue was dissolved in 50 ml of ethyl acetate and washed with 1N HCl (3 × 20 ml), 5%, m/v, $NaHCO_3$ (3 × 20 ml), and finally with water (3 × 20 ml). The organic phase was dried over Na_2SO_4 and evaporated under reduced pressure to yield a crude oil, that was purified by silica gel column chromatography (mobile phase: petroleum ether/ethyl acetate, 80:20, v/v).

A solution of 1.0 mmol of the *N*-BOC-protected anilide in 100 ml of chloroform was cooled to 0–5 °C and saturated with HCl gas. Removal of the solvent under reduced pressure gave the crude compound as a solid, which was recrystallized from ethanol and ethyl acetate to give a colorless solid of *N*-(3-hexyloxyphenyl)piperidine-3-carboxamide as hydrochloride salt.

*2.1.4. Synthesis of 1-alkyl-N-(4-hexyloxyphenyl)piperidine-3-carboxyamides (**28a**, **28b**)*

*2.1.4.1. Step 1. Synthesis of N-(4-hexyloxyphenyl)nicotinamide (**26**).* A mixture of nicotinic acid (1.0 g, 8.1 mmol), thionyl chloride (0.70 ml, 8.1 mmol) and redistilled pyridine (1.26 ml, 16.2 mmol) in 30 ml of dry toluene was heated at 100 °C for 1 h. After cooling to room temperature, 4-hexyloxyaniline (1.56 g, 8.1 mmol) was added and the reaction mixture stirred at 60 °C for 3 h and at 100 °C for 1 h. After cooling, the crude solid product was filtered-off and dissolved in chloroform (25 ml). The organic solution was washed with 10%, m/v, solution of Na_2CO_3, dried (Na_2SO_4) and concentrated under reduced pressure to afford 2.26 g of the desired amide compound.

*2.1.4.2. Step 2. 1-methyl-3-{[(4-hexyloxyphenyl)amino] carbonyl}pyridinium iodide (**27a**).* To a cooled solution of **26** (0.50 g, 1.7 mmol) in 4 ml of absolute ethanol, methyl iodide (0.4 ml, 6.8 mmol) in 2 ml of acetone was added dropwise. After refluxing for 3 h and cooling to room temperature, the crude solid product was filtered-off and recrystallized from absolute ethanol to afford 0.77 g of pale yellow crystals.

*2.1.4.3. 1-propyl-3-{[(4-hexyloxyphenyl)amino]carbonyl} pyridinium iodide (**27b**).* Compound **26** (0.50 g, 1.7 mmol) was refluxed with 10.0 ml of 1-bromopropane for 60 h and cooled to room temperature. The crude solid product was filtered-off and recrystallized from absolute ethanol to afford 0.55 g of a pale yellow crystals.

*2.1.4.4. Step 3. 1-methyl- (**28a**) and 1-propyl N-(4-hexyloxyphenylpiperidine-3-carboxamide hydroiodide (**28b**).* A suspension of 4 mmol of **27a** (1.9 g), or **27b** (0.51 g), and 150 mg of PtO_2 in 30 ml of ethanol and 10 ml of water was stirred under hydrogen atmosphere at 60 psi until TLC revealed disappearance of the starting material, followed by the removal of the catalyst and the solvent. Recrystallization of the residues from ethanol gave 1.2 g of **28a** hydroiodide as yellow crystals.

Compound **28b** (highly hygroscopic) was transformed into the corresponding hydrochloride salt (0.22 g), that was crystallized from ethanol.

*2.1.5. Synthesis of N-(piperidin-3-ylmethyl)-N-(4-hexyloxyphenyl)amine (**25**)*

To a solution of 4-hexyloxyanilide of nipecotic acid **17c** (0.50 g, 1.6 mmol) in dioxane, borane–trimethylamine complex (0.58 g, 8.0 mmol) was added, and the mixture was refluxed overnight. After removal of the solvent, the oil residue was dissolved in 50 ml of chloroform and the organic layer washed with saturated $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated under reduced pressure, affording 0.20 g of 25 as brown oil. MS *m*/*z* 290 (M^+, base).

2.2. In vitro antiplatelet activity

Human blood was obtained from healthy volunteers (25–45 years of age), who had not ingested any platelet inhibitory drug for at least 1 week prior to donation. All subjects gave written informed consent about this study, according with the Helsinki and Tokyo declarations and the European Community Guidelines. Blood and blood products were handled in plastic ware, whereas siliconized glass cuvettes and stir bars were used in the aggregation assay.

Human platelet-rich plasma (PRP) was obtained from the supernatant 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 800 rpm for 15 min at 21 °C. Platelet-poor plasma (PPP) was obtained after centrifugation of the venous blood at 3200 rpm for 10 min. Platelet counts were adjusted to the constant value of 230,000 $\mu l^{-1}$by using PPP.

Aggregation was measured by the turbidimetric method of Born (Born, 1962), using a four-channel aggregometer (PACKS-4, Helena Laboratories S.p.A., Beaumont, TX, USA). The transmittance of PPP was taken as 100% aggregation.

PRP (250 μl) was pre-incubated with the test compounds (5 μl solutions; final concentrations in the PRP solutions ranging from 50 to 800 μM) or with controls (to eliminate the effect of the solvent on the aggregation and release reaction of platelets, the final concentration of DMSO was fixed at 0.5%, v/v) at 37 °C for 5 min., during which the suspension was stirred at 800 rpm. Then, 50 μl plasma isotonic solution (0.9%, m/v, NaCl) containing ADP (10 μM final concentration) or arachidonic acid (AA, 1.5 mM final concentration), both purchased from Helena Biosciences Europe (Sunderland, UK), was added to the stirred sample and the change in transmittance at 640 nm was recorded for 5 min. The control cuvette containing the vehicle-treated PRP followed the same sequence of events. The above concentrations of ADP and AA are well established to induce a full response in routine platelet aggregation tests. All the aggregation experiments were finished within 3 h from the blood collection, whereas each series of data for dose–response curves in triplicate was collected within 1 h.

Inhibition of aggregation was expressed as a percent difference of the maximum aggregatory response. For each compound dose–response relations were determined in a concentration interval ranging from 50 to 800 μM (for the most active compounds also 25 μM concentration was tested), and IC_{50} values (concentration effecting 50% inhibition of aggregation), obtained by nonlinear (sigmoid) or linear regression of aggregation inhibition (IA%) on log concentration of test compounds ($r^2 > 0.75$), were reported as means ± S.E.M. of at least three different determinations.

2.3. Measurement of intraplatelet calcium ($[Ca^{2+}]_i$) mobilization

Measurements of intraplatelet Ca^{2+} mobilization were performed by using the fluorescent dye for Ca^{2+} fura-2-acetoxymethyl ester (Fura-2 AM) according to a previously described method (De Candia et al., 1999; De Cristofaro et al., 1995).

Blood was obtained from healthy volunteers who denied drug ingestion 7 days prior to phlebotomy, and drawn into plastic tubes containing trisodium citrate as anticoagulant. Whole blood was centrifuged at 800 rpm for 15 min to obtain platelet rich plasma (PRP). Loading of platelets with Fura-2-AM was carried out by incubating PRP with 5 μM Fura-2 AM at 37 °C for 30 min. Fura-2 AM had been previously dissolved in DMSO (10 mM concentration) and kept at −80 °C until use.

At the end of incubation, platelets were gel filtered on a Sepharose 2B column (20 cm × 1.6 cm), equilibrated with 10 mM Hepes, 135 mM NaCl, 5 mM KCl, 5.5 mM glucose, 0.2% bovine albumin, pH 7.4. Platelet gel filtration allowed us to both obtain a plasma-free platelet suspension and eliminate Fura-2 AM excess. Platelet count was determined with a Dasit K 4500 Cell Counter, and adjusted to 150,000 μl^{-1}.

Changes in fluorescence intensity were measured by means of a Perkin–Elmer LS5B spectrofluorometer connected to a High-Tech Scientific (Salisbury, UK) SFA-II Rapid Kinetic Accessory. The excitation and emission wavelenghts were 340 and 500 nm with 10 and 5 nm bandwidths, respectively. The spectrofluorometer was connected to a computer which received signals at 100 ms time intervals.

The influx of external calcium was avoided by adding 1 mM EDTA to platelet suspensions before agonist addition. EDTA per se had no effect on intraplatelet fluorescence (not shown). Platelets were incubated with 32–280 μM test compound **17c** (or 0.5%, v/v, DMSO in control curves) for 10 min before platelet stimulation by 10 μM ADP. As previously reported (De Cristofaro et al., 1995), in these conditions the fluorescence variations after agonist addition measured the changes of intraplatelet calcium concentrations due only to mobilization from internal storages. The maximum value of fluorescence was taken as the parameter to evaluate the effect of different **17c** concentrations.

2.4. Lipophilicity parameters

Lipophilicity was computationally estimated by using the CLOG P software (CLOG P for Windows Version 4.73, BioByte Corp., Claremont, CA, USA).

The lipophilicity of nipecotic acid derivatives (Tables 1 and 2) was determined by a reversed-phase HPLC method. Retention data were measured using a Symmetry C18 (4.6 mm × 150 mm i.d. 5 μm particles) column (Waters Assoc., Milford, MA, USA) as the nonpolar stationary phase, at regular increments of the volume fraction of methanol (φ) in the aqueous mobile phase (0.040 M phosphate buffer, pH 5.8). All the retention data were collected at a temperature of 25 ± 0.2 °C (thermostated column and eluent reservoir), the flow-rate of 1.0 ml/min and at 254 nm on a Waters HPLC 1525 multisolvent delivery system, equipped with a Waters 2487 variable wavelength UV detector (Waters Assoc.).

Capacity factors (k') of each compound at different mobile phase compositions (0.05-increments of methanol φ) were calculated as: $k' = (t_R - t_0)/t_0$, where t_R is the retention time of the solute and t_0 is the column dead time, measured as the solvent front. The solutes were divided into three groups, depending on their lipophilicity as calculated by the CLOG P software. In the case of more hydrophilic compounds (Clog $P < 2$), the capacity factors (k') at $\varphi = 0.30$ of methanol in the mobile phase were directly measured. For the other solutes, k' values were obtained within different ranges of methanol φ. The $\log k'$ values increased linearly ($r^2 > 0.95$) with decreasing methanol volume fraction, and $\log k'$ at $\varphi = 0.30$ were calculated by using the linear relationship: $\log k' = \log k'_w - S\varphi$ (S, the slope, is a constant for the solute–eluent combination). In a few cases, when the relationship between $\log k'$ and φ deviated from linearity at

the highest φ values (an evidence of the so-called mixed retention mechanism) the linear extrapolation was performed on part of the points, i.e. those with lowest organic methanol concentration.

Both, computational and experimental lipophilicity values are reported in Tables 2 and 3.

3. Results and discussion

3.1. Chemistry

New alkoxyphenyl piperidine-3-carboxamides (**17a**, **17b** and **18**, Table 2) were synthesized according to Scheme 1. 1-(*tert*-Butyloxycarbonyl)-piperidine carboxylic acids, prepared by a reported method (Bonina et al., 1999), were condensed with para or meta substituted anilines, using diisopropylethyl amine (DIEA) as proton scavenger, and dicyclohexylcarbodiimmide (DCC) and 1-hydroxybenzotriazole hydrate (HOBt) as coupling reagents, in dry dichloromethane. Deprotection of the *N*-BOC intermediates with HCl gas yielded the amide derivatives as hydrochloride salts. The amide carbonyl of **17c** was reduced to a methylene group in **25** using excess borane–trimethylamine complex. 4-Hexyloxybenzylamine (**22**), necessary to synthesize amide **24**, was obtained through alkylation of 4-hydroxybenzonitrile with *n*-hexyl bromide, to give compound **21**, followed by catalytic (Pd/C) hydrogenation of the nitrile group.

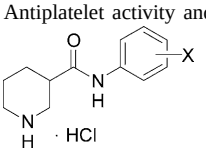
The ring-opened analog of piperidine-3-carboxamide **30** and its homologs **29** and **31** were prepared through the same procedures outlined in Scheme 1.

Nipecotamides **28a** and **28b** bearing a methyl and an *n*-propyl group on piperidine nitrogen, respectively, were synthesized according to the pathway shown in Scheme 2. Nicotinic acid was converted into the acid chloride, followed by acylation of 4-hexyloxyaniline. The resulting amide was treated with the alkyl (methyl and *n*-propyl) iodides to provide the pyridinium salts **27a** and **27b** which, upon hydrogenation (PtO_2/H_2), afforded **28a** and **28b**.

3.2. Platelet activation inhibitory activity

The antiplatelet effects of 10 newly synthesized piperidine-3-carboxamide derivatives and analogs (**17a**, **17b**, **18**, **24**, **25**, **28**–**31**) were tested on the in vitro ag-

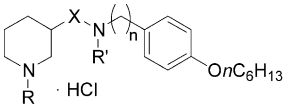
Table 2
Antiplatelet activity and lipophilicity of piperidine-3-carboxamide derivatives



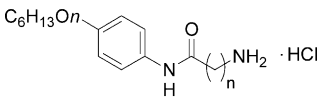
Compound	X	IC_{50} (μM)[a] or %inhibition at 100 μM	N[b]	pIC_{50}	Lipophilicity	
					log P[c]	log k'[d]
2[e]	4-H	540	5	3.27	1.53	1.13
3[f]	3-Br	(0%)	3		2.65	2.09
4[f]	4-Br	162	3	3.79	2.65	2.06
5[f]	3-NO_2	(0%)	3		1.82	1.33
6[e]	4-NO_2	236	5	3.63	1.82	1.41
7[f]	4-CF_3	372	3	3.43	2.86	2.24
8[f]	4-CH_3	270	3	3.57	2.03	1.56
9[f]	3-OCH_3	(0%)	3		1.60	1.29
10[e]	4-OCH_3	983	5	3.01	1.60	1.13
11[f]	4-$NHSO_2CH_3$	(23 ± 3%)	3		0.34	0.41
12[f]	4-$COCH_3$	(0%)	3		1.44	1.03
13[f]	4-$C(CH_3)_3$	269	3	3.57	3.35	2.65
14[f]	4-nC_7H_{15}	102	3	3.99	5.20	4.44
17a	2-OnC_6H_{13}	144	3	3.84	3.66	3.20
17b	3-OnC_6H_{13}	113	3	3.95	4.25	3.74
17c[e]	4-OnC_6H_{13}	75	7	4.13	4.25	3.59
18	3-O$nC_{11}H_{23}$	103	3	3.99	6.89	5.63
19[f,g]	4-OnC_6H_{13}	141	3	3.85	3.49	3.11

[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) was then added. Activity is expressed as IC_{50} or percent of inhibitory response at 100 μM concentration for less active compounds.
[b] Number of individual determinations.
[c] *n*-Octanol–water partition coefficient calculated by CLOG P software version 4.73 (Biobyte, Claremont, CA, USA).
[d] RP-HPLC capacity factor extrapolated at 30% methanol volume fraction in aqueous mobile phase.
[e] Compounds previously reported (de Candia et al., 2003) and re-tested in this study.
[f] Compounds previously reported (de Candia et al., 2003).
[g] *N*-[(4-hexyloxyphenyl)]piperidine-4-carboxamide hydrochloride.

Table 3
Antiplatelet activity and lipophilicity of derivatives and ring-opened analogs of 4-hexyloxyphenyl nipecotamide



20, **24**, **25**, **28a-b**



29-31

Compound	*R*	X	*R′*	*n*	IC_{50} (μM)[a] or %inhibition at 100 μM	*N*[b]	pIC_{50}	Lipophilicity	
								log *P*[c]	log *k′*[d]
20[e]	H	CO	CH_3	0	120	3	3.92	4.31	3.72
24	H	CO	H	1	117	3	3.93	4.05	3.53
25	H	CH_2	H	0	115	3	3.94	4.62	3.50
28a[f]	CH_3	CO	H	0	80	3	4.10	4.66	4.34
28b	nC_3H_7	CO	H	0	150	3	3.83	5.72	3.64
29				2	(17 ± 6%)	3		3.32	3.11
30				3	(32 ± 3%)	3		3.66	3.27
31				4	(26 ± 5%)	3		3.58	3.49

[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) was then added. Activity is expressed as IC_{50} or percent of inhibitory response at 100 μM concentration for less active compounds.
[b] Number of individual determinations.
[c] *n*-Octanol–water partition coefficient calculated by CLOG P software version 4.73 (Biobyte, Claremont, CA, USA).
[d] RP-HPLC capacity factor extrapolated at 30% methanol volume fraction in aqueous mobile phase.
[e] Compounds previously reported (de Candia et al., 2003) and re-tested in this study.
[f] Tested as hydroiodide salt; all the other compounds were tested as hydrochloride salts.

gregation of human platelet-rich plasma (PRP) triggered by 10 μM ADP, using a turbidimetric method. Four previously reported nipecotamides (**2**, **6**, **10**, and **17c**) were re-tested in this study, whereas different concentrations of 4-hexyloxyanilide **17c**, taken as a reference compound, were included in each series of determinations in order to continuously ascertain the reliability of the experimental methodology. Based on our previous study (de Candia et al., 2003), compounds showing inhibitory effects at 100 μM concentration significantly different from controls ($P < 0.001$) were subjected to a screening of dose-dependent effects on the aggregation of human PRP and concentrations inhibiting platelet aggregation by 50% (IC_{50}) were determined. The in vitro inhibitory activities are summarized in Table 2 (congeners) and Table 3 (analogs and derivatives) as the average IC_{50} values or, for weak inhibitors, percent of inhibition (mean ± S.E.M.) at 100 μM from at least three individual determinations. A number of previously reported data have been also included in the tables to provide structure–activity relations.

No compound displayed effects stronger than those measured with 4-O*n*C_6H_{13} nipecotanilide **17c** ($IC_{50} = 75$ μM), which on the other hand had shown a degree of inhibitory activity similar to that of aspirin in adrenaline-induced PRP aggregation (de Candia et al., 2003). The flexible and lipophilic *n*-hexyloxy substituent in the para position (**17c**) displayed slightly better effects than when it was in the meta (**17b**) and in the ortho-position (**17a**). The ortho-substituted congener **17a** was in fact one half as active as the para-substituted **17c**. Homologation of phenyl amide **17c** to benzyl amide **24** did not afford any significant improvement in antiplatelet activity. The ring-opened analogs, i.e. the 4-hexyloxyphenyl amide of β-alanine **29** and its higher homologs **30** and **31**, were by contrast significantly less active than **17c**.

As far as the effects of the piperidine nitrogen alkylation are concerned, our data showed that a methyl (**28a**) is tolerated, whereas an *n*-propyl (**28b**) halves the aggregation inhibitory effects.

The functional importance of the amide carbonyl in **17c** was also investigated, and the biological data showed a slight but significant decrease in activity when it was reduced to a CH_2 group in **25**. This decrease was less strong than that previously found by Feng et al. (1992) with the bis-nipecotamides **1** (the resulting amine was about 1/40th as active as its carbamoyl derivative), most likely because of the different degree of basicity of the amines obtained from **17c** (aromatic) and **1** (aliphatic). Due to its low basicity, the amine compound **25** should be predominantly present in its mono-protonated form at physiological pH (calculated distribution coefficient, $\log D = 1.41$), whereas the amine derivative resulting from the carbonyl reduction of the bis-amide **1** should be largely present in its tri-cationic form ($\log D = -0.65$), most likely having limited penetration through the platelet plasma membrane.

The effects of three piperidine-3-carboxamides, namely **17b**, **17c** and **24**, on platelet aggregation induced by AA were also evaluated in order to investigate whether a platelet signaling pathway, different from that used by ADP, i.e. the TxA_2-mediated platelet activation and aggregation pathway, could be affected by lipophilic nipecotamides. As previously demonstrated by others (Burke et al., 2003) and confirmed in our preliminary experiments, 1.5 mM is the

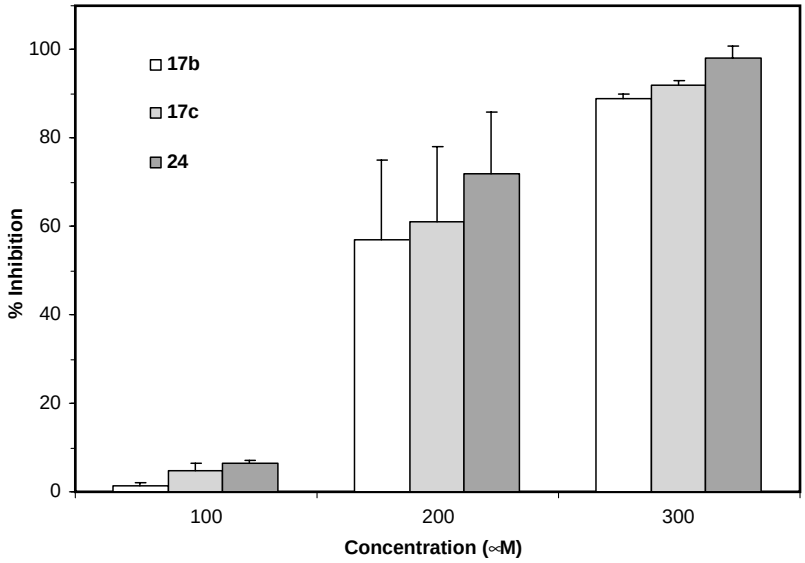


Fig. 2. Bar diagram of the inhibitory effects (%) at 100, 200 and 400 μM concentrations on platelet aggregation induced by arachidonic acid (1.5 mM). Data are reported as means ± S.E.M. ($n = 3$); control amplitude (92.4 ± 1.00) was set as 100% aggregation. The tested compounds resulted inactive at 50 μM concentration.

concentration of AA that induces a maximal aggregation in more than 90% of healthy subjects. As shown by Fig. 2, all the compounds tested inhibited AA-induced platelet aggregation in a concentration-dependent manner, and their IC_{50} should be around a concentration of 200 μM.

Finally, the effects of different concentrations of 4-hexyloxyanilide of nipecotic acid **17c** on intracellular calcium ($[Ca^{2+}]_i$) mobilization were measured using Fura-2 AM as a fluorescent dye for intracytoplasmic calcium and spectrofluorometric measurements of fluorescence variations induced by ADP (De Candia et al., 1999), and the results are graphically illustrated in Fig. 3.

In Fura-2-loaded platelets, ADP (10 μM) increased the fluorescence intensity of the calcium sensitive dye corresponding to an increase in intracellular Ca^{2+} concentration; the rise was short-lived, and the fluorescence declined towards the resting level in less than 2 min. As shown in Fig. 3, the intracellular calcium mobilization induced by ADP was 50% inhibited by **17c** at doses around 100 μM. Since calcium mobilization by agonists is a very early biological response, our results suggest that the inhibitory activity of **17c** would occur at the initial phases of the signal transduction processes, according to the hypothesized mechanism of nipecotamides' action (Dillingham et al., 1989; Feng et al., 1992; Guo et al., 2000).

3.3. Lipophilicity

The partitioning properties of all the derivatives in Tables 2 and 3 were investigated by measuring capacity

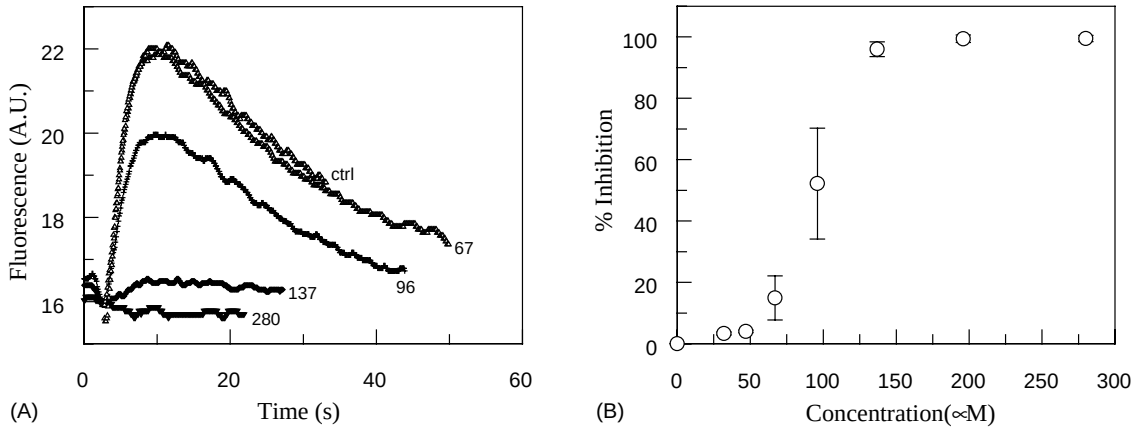


Fig. 3. Concentration-dependent effects of 4-*n*-hexyloxyphenyl amide of nipecotic acid (**17c**) on the increase in intraplatelet calcium concentration induced by ADP (10 μM). (A) For the sake of clarity, only four tracings at the reported compound concentrations (μM) are shown. (B) The maximum fluorescence value for each compound concentration was measured and reported as % inhibition (mean ± S.E.M. of three experiments).

factors ($\log k'$) in RP-HPLC (Altomare et al., 1993; Altomare et al., 1994). For eight compounds with Clog P values ranging from 0.3 to 2.0, the isocratic capacity factors at 30%, v/v, methanol concentration in aqueous mobile phase were measured, while for the others, retention data were determined at 5% increments of the volume fraction of methanol in the aqueous mobile phase ($0.3 < \varphi < 0.8$), and $\log k'$ values at 30% methanol volume fraction obtained through extrapolation of the linear relationships between $\log k'$ and φ. With the noteworthy exception of the 1-propyl derivative **28b**, which showed a lower-than-expected lipophilicity (the difference was more than one log unit), all the compounds reported in Tables 2 and 3 afforded the following statistically good linear relationship between $\log k'$ and calculated $\log P$:

$$\log k' = 0.88(\pm 0.03)\text{Clog}\,P + 0.12(\pm 0.10) \quad (1)$$

$$n = 26, \qquad r^2 = 0.974, \qquad s = 0.215, \qquad F = 860$$

where n represents the number of data points, r^2 the squared correlation coefficient, 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.

Replacing Clog P in Eq. (1) with calculated distribution coefficients ($\log D$) at the apparent pH where the RP-HPLC capacity factors were determined, i.e. pH 5.8 (pK_a values were estimated by the ACDLabs program), we observed no appreciable change in the statistics and in the slope value, but only in the intercept of Eq. (1), indicating that protonation at the piperidine nitrogen should equally affect the RP-HPLC retention of all the piperidin carboxamides examined.

In an attempt to understand the anomalous behavior of the 1-*n*-propyl nipecotamide derivative **28b** with respect to Eq. (1), we investigated its conformation-dependent lipophilicity as compared with the lower homologs **28a** (1-CH_3) and **17c** (1-H). High-temperature molecular dynamics (MD) simulations[1] performed on the protonated species of the lipophilic nipecotanilides allowed us to generate a distribution of low-energy conformers with a large spread of lipophilicity, as assessed by the calculation of the molecular lipophilicity potential (MLP)[2] over the water-accessible surface areas.

The derivatives adopted several conformations during the simulation, which oscillated between different folded forms (F), with lower virtual $\log P$, and extended forms (E) with higher virtual $\log P$.

The global minimum of the 1-*n*-propyl derivative **28b** showed F geometry with a virtual $\log P$ of 3.11, whereas the highest $\log P$ (3.76) was found to be associated with the most extended (E) conformers, revealing a rather regular increase in lipophilicity as the molecular folding becomes increasingly less pronounced. The difference between the relative energies of the global minimum F and the most extended E conformational states was found to be equal to 2.3 kcal/mol. Using the same MD tactics, the lower homologs **17c** and **28a** also showed a folding-dependent lipophilicity. The lowest-energy conformers displayed F geometries with a $\log P$ of 1.79 (**17c**) and 2.01 (**28a**), whereas the most extended conformers resulted the most lipophilic ones, with virtual $\log P$ equal to 2.25 and 2.87, respectively. The ΔE values of the $F \rightleftarrows E$ interconversion were equal to 1.3 and 0.40 kcal/mol for **17c** and **28a**, respectively, both values being lower than that associated with **28b**. These data suggest that 1-*n*-propyl anilide **28b** may prefer folded, less lipophilic, conformations, more than its lower homologs **17c** and **28a**, which should undergo transitions between F and E conformers through lower energy barriers.

Even though the comparison between calculated and experimental lipophilicity parameters in absolute values would not make sense, due to the different lipophilicity fragmental constants adopted in the Clog P and MLP calculation systems and the different scale of $\log k'$, the preference of **28b** for less lipophilic molecular geometries may explain not only its anomalous behavior with respect to Eq. (1), but also (at least in part) the loss of activity compared with the lower homologs **17c** and **28a**, which by contrast showed very close activity values.

The plot in Fig. 4, built up for eighteen compounds with finite antiplatelet activity data (pIC_{50}, i.e. $\log 1/IC_{50}$), shows a nonlinear dependence of antiplatelet activity upon lipophilicity, as it had already been demonstrated for other series of antiplatelet agents (Feng et al., 1992; Tanaka et al., 1994; Tanaka and Fujiwara, 1996), and the following parabolic relationship was calculated, which explains about 80% of the variance in the activity data:

$$pIC_{50} = 0.52(\pm 0.13)\log k' - 0.05(\pm 0.02)(\log k')^2 + 2.75(\pm 0.18) \quad (2)$$

$$n = 18, \qquad r^2 = 0.783, \qquad s = 0.148, \qquad F = 27.00$$

Using Clog P instead of $\log k'$ afforded a statistically poorer equation ($r^2 = 0.738$, $s = 0.162$), whereas adding to the lipophilicity parameters bulkiness (e.g. molar refractivity, molar volume) and electronic (e.g. sigma constants) descriptors did not improve the statistics. Actually, our activity and lipophilicity data also fitted the Kubinyi bilinear model (Kubinyi, 1993) quite well, yielding an equation (not shown) with statistics comparable with those of Eq. (2)

[1] Sybyl (version 6.8) from Tripos Associates Inc., St. Louis, MO, USA Energy minimizations: Tripos Force Field, Gasteiger–Marsili charges, dielectric constant $\varepsilon = 1$ and a convergence criterion of 0.001 kcal mol^{-1} Å^{-1}. MD simulations: 100 ps (1.0 fs steps) at 2000 K (frame data stored every 0.05 ps); 200 randomly selected conformations were energy-minimized, matched using the rigid fit option and clusterized based on geometrical criterion (calculated r.m.s.d.) by the NMRCLUST program. All the calculations were run on Silicon Graphics workstations (SGI O2 R10000).

[2] The molecular lipophilicity potential (MLP) and virtual $\log P$ (Gaillard et al., 1994; Carrupt et al., 1995) were calculated for the minimum-energy structure of each cluster identified, using the CLIP program (version 1.0).

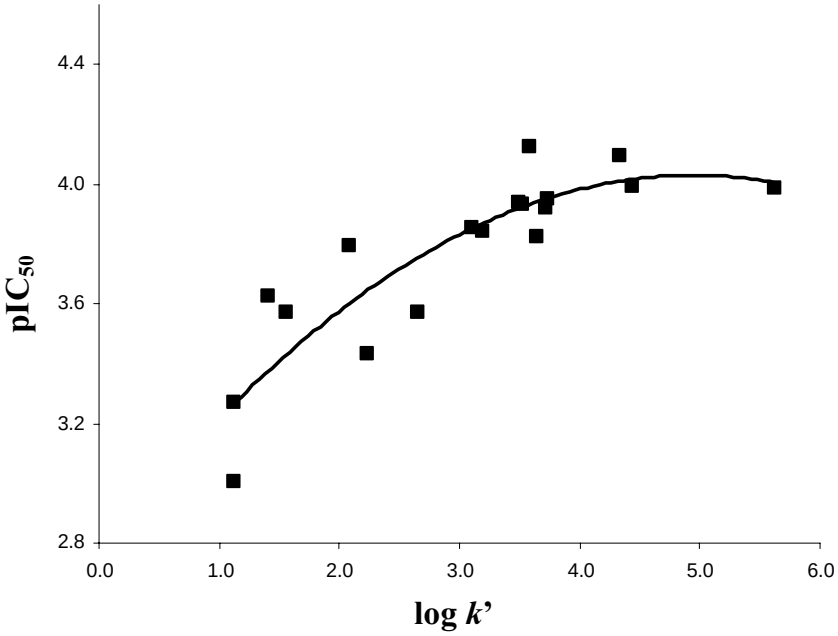


Fig. 4. Parabolic relationship between platelet aggregation inhibitory activity (pIC_{50} = −log of compound concentration which inhibits ADP-induced aggregation by 50%) and lipophilicity as assessed by RP-HPLC of nipecotamide derivatives (log k').

($r^2 = 0.786$, $s = 0.151$). Therefore, based on the statistical parameters we can not decide unequivocally if the dependence of the activity on lipophilicity is parabolic or bilinear, whereas the dominant role played by lipophilicity in modulating the platelet aggregation inhibitory activity of the piperidine-3-carboxamide derivatives is clearly demonstrated. The mathematical model accounted for by Eq. (2) may well reconcile with their postulated mechanism of action in which (i) penetration through the lipid bilayer of the platelet membrane should be the rate-limiting step and (ii) interaction with the anionic phosphoinositides of the platelet membrane would trigger a number of processes leading to the inhibition of platelet aggregation. In both events micelle formation and/or limited solubility of more lipophilic analogs, which constitute reasonable explanations for the nonlinear lipophilicity–activity relationships observed, should be involved. Such mechanistic aspects are being examined.

4. Conclusions

Based on our previous studies and the results presented herein, simple anilides of nipecotic acid bearing lipophilic substituents on the phenyl ring inhibit platelet aggregation induced by ADP. The activity was structure-dependent, and the 4-*n*-hexyloxy group proved to be the optimal substituent on the phenyl group. For the most active nipecotamide (**17c**) within the molecular set investigated, we also proved inhibition of the PRP aggregation triggered by AA and ADP-induced intracellular calcium mobilization. AA induces in vitro and in vivo the formation of TxA_2, a physiological second messenger of platelet aggregation. Both ADP and AA released from platelets act as soluble factors that recruit more platelets in the vessel for the formation of a stable aggregate. Thus, the final effect of nipecotamides is the inhibition of two signaling pathways contributing to the amplification of platelet aggregation. While the exact mechanism for inhibition of intraplatelet calcium mobilization by this series of compounds would deserve further investigation, the following relevant structure–activity relationships were inferred from our data: (1) the impact of hydrophobicity on antiplatelet activity is demonstrated by a nonlinear (parabolic or bilinear) relationship between activity data and lipophilicity parameters; (2) quasi-isolipophilic isomers (i.e. isonipecotanilides and pipecolinanilides) and ring-opened analogs (e.g. anilide of β-alanine) exhibited lower inhibitory effects compared to the corresponding nipecotanilides on platelet aggregation; (3) alkylation of the piperidine nitrogen has no effect (methyl group) or decreases (*n*-propyl group) the activity by a factor of approximately 2.

Acknowledgements

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