Searching for Balanced Hybrid NO-Donor 1,4-Dihydropyridines with Basic Properties

Searching for Balanced Hybrid NO-Donor 1,4-Dihydropyridines with Basic Properties

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Purpose. Model compounds containing NO-donor furoxan moieties at the 3-positioned basic lateral chain of **1**, a 1,4-dihydropyridine related to nicardipine, were synthesized in order to study their vasodilating activity as well as their basic and lipophilic behaviour.

Methods. All the compounds were obtained by a modified Hantzsch approach. Potentiometry was used to determine pK_a and lipophilicity descriptors. The furoxan 4-aryl-1,4-dihydropyridines were assessed for their ability to release nitrite, in the presence of a large excess of cysteine, by the Griess reaction. Vasodilating activity of the products in the absence and in the presence of ODQ, a well-known guanylate cyclase inhibitor, was evaluated on rat thoracic aorta.

Results. The compounds display low basicity values and for this reason their log Ds at physiological pH are identical to the log Ps of the neutral forms. Products **2**, **3** display vasodilating action principally dependent on their Ca^{2+}-antagonist properties, whereas **4** behaves as a well-balanced hybrid with mixed Ca^{2+}-channel blocker and NO-dependent vasodilator activities.

Conclusions. Nitrogen containing lateral chain at the 3-position of **1** is a suitable molecular region to be modified in order to obtain well-balanced furoxan NO-donor 1,4-DHPs. This manipulation produces a decrease in the basicity. General analysis of pK_a and lipophilicity descriptors of these new DHPs suggest that molecular flexibility could influence both their basicity and log P^I.

KEY WORDS: 1,4-Dihydropyridines; nitric oxide (NO); NO donors; hybrid drugs; furoxans.

INTRODUCTION

Recently a research program was started in our laboratory aimed at obtaining new "hybrid" 1,4-dihydropyridines (1,4-DHPs) endowed with mixed nitric oxide (NO)-like and Ca^{2+}-channel antagonist vasodilating properties. Nifedipine was first selected as a model for the development of these new drugs. We substituted the *o*-nitro group with *o*- and *m*-positioned furoxan moieties at the 4-phenyl ring, gifted with different NO-releasing property (1). In a further study these same substructures were introduced at the 3-positioned chain of the lead (2).

We have now undertaken the study of basic NO-donor 1,4-DHPs obtained by introducing NO-donor furoxan systems on the nitrogen lateral chain of product **1**, whose structure is related to nicardipine. The furoxan ring bears suitable substituents chosen for modulating its ability to release NO to obtain well-balanced hybrids. The NO-donor moieties were joined to the nitrogen in the lateral chain by a spacer selected on the basis of the synthetic accessibility of the final models.

In this preliminary communication we report synthesis, NO-release, and vasodilating activity of the new products **2**, **3**, and **4**. We also evaluated their pK_a and log D, tools useful for the next pharmacokinetic study.

MATERIALS AND METHODS

Synthesis

Melting points were measured on a Büchi 530 capillary apparatus and are uncorrected. Melting points with decomposition were determined after introducing the sample into the bath at a temperature 10°C lower than the melting point. A heating rate of 3°C min^{-1} was used. Infrared spectroscopy, ^1H and ^{13}C nuclear magnetic resonance at 200 MHz and 50 MHz, respectively, and mass spectroscopy routinely checked the compounds. All the spectra were in accordance with the expected structures. Column chromatography was performed on silica gel (Merck Kieselgel 60, 230–400 mesh ASTM, Merck, Milan) with the indicated solvent system. Petroleum ether 40°–60°C (PE) was used. Solvent removal was achieved under reduced pressure at room temperature. Elemental analyses of the new compounds were performed by REDOX (Cologno Monzese) and the results were within ±0.4% of the theoretical values. Intermediates **1** (3), **5**, **6** (1), **7** (5), and **8** (4), were synthesized according to procedures described in the literature. Compound **10**, 3-nitrobenzaldehyde, and methyl 3-aminocrotonate were commercial reagents (Aldrich Chemical Co., Milwaukee, WI).

*General Procedure for the Synthesis of 1,4-DHPs **2, 3***

The appropriate 4-bromomethylfuroxan **5** or **6** (4.8 mmol) was added to a solution of **1** (1.86 g, 4.8 mmol) in a mixture of acetone (30 ml) and 0.5 N $KHCO_3$ (20 ml). The solution was stirred for 6 h and then was neutralized with 1N HCl; the acetone was evaporated and the aqueous solution was extracted with ethyl acetate. The dried organic phases were evaporated, and the residue was purified by flash-chromatography. The purified fractions were dissolved in HCl saturated methanolic solution. Solvent removal gave the final product as a hydrochloride. Chromatographic solvents, yields, melting points, and analytical data were as follows:

*Methyl 2-(N-methyl-N-(3-methylfuroxan-4-yl)methyl)-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate hydrochloride (**2**).* Eluent: CH_2Cl_2/MeOH 9.7/0.3; yield 44%; softening at 79°C decomposed at 110°C. Anal. ($C_{23}H_{27}N_5O_8 \cdot HCl \cdot 0.5H_2O$) C, H, N.

*Methyl 2-(N-methyl-N-(3-carbamoylfuroxan-4-yl)-methyl)aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridindicarboxylate hydrochloride (**3**).* Eluent: CH_2Cl_2/MeOH 9.9/0.1→9.7/0.3; yield 84%; softened at 135°C and then slowly decomposed until 151°C. Anal.($C_{23}H_{26}N_6O_9 \cdot HCl \cdot H_2O$) C, H, N.

*4-(2-(N-(2-Hydroxyethyl)-N-tert-butoxycarbonylamino)-ethyloxy)-3-phenylsulfonylfuroxan (**9**)*

Fifty percent NaOH (3.28 g, 41 mmol) was added dropwise to a solution of **7** (12.44 g, 34 mmol) and **8** (7.02 g, 34 mmol) in THF (100 ml) at −5°C. The mixture was stirred at

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–15°C for 2.5 h, then phosphate buffer (pH 5.4, 0.5 M, 100 ml) was added and the solution obtained was extracted with CH_2Cl_2. The dried organic phases were evaporated and the residue was purified by flash-chromatography (eluent: PE/EtOAc 8/2). The purified product was an unstable oil that was immediately used for the preparation of the intermediate **11**. The yield was quantitative.

*N-(2-(3-Phenylsulfonylfuroxan-4-yloxy)ethyl)-N-tert-butoxycarbonyl-2-aminoethyl acetoacetate (**11**)*

A solution of **9** (2.06 g, 4.8 mmol) and 2,2,6-trimethyl-4H-1,3-dioxin-4-one (**10**) (0.68 ml, 4.8 mmol) in toluene (20 ml) was refluxed for 1 h. Solvent removal gave a residue, which was purified by flash chromatography (eluent: PE/EtOAc 7/3). Yield 60%, oil. Anal. ($C_{21}H_{27}N_3O_{10}S$) C, H, N.

*Methyl N-(2-(3-phenylsulfonylfuroxan-4-yloxy)ethyl)-N-tert-butoxycarbonyl-2-aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate (**12**)*

A solution of **11** (3.50 g, 6.8 mmol), 3-nitrobenzaldehyde (1.03 g, 6.8 mmol), and methyl 3-aminocrotonate (0.78 g, 6.8 mmol) in 2-propanol (80 ml) was refluxed for 10 h. Solvent removal gave a residue, which was purified by flash chromatography (eluent: PE/EtOAc/MeOH 7/2.5/0.5). The product obtained was a yellow solid. Yield 51%, softened at 79°C decomposed at 105°C. Anal.($C_{33}H_{37}N_5O_{13}S$) C, H, N.

*Methyl 2-(N-(2-(3-phenylsulfonylfuroxan-4-yloxy)ethyl)aminoethyl 1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5-pyridinedicarboxylate hydrochloride (**4**)*

HCl saturated diethyl ether was added to a solution of **12** in dry CH_2Cl_2 and the mixture was stirred for 48 h. Solvent removal gave a residue that was triturated with dry diethyl ether, filtered, and dried. Yield 66%; darkening at 115°C, decomposed at 130°C. Anal. ($C_{28}H_{29}N_5O_{11}S{\cdot}HCl{\cdot}0.5H_2O$) C, H, N.

pH-Metric Approach to Obtain pK_a and Log P

Potentiometric titrations of compounds (**1**–**4**) were performed with the GlpKa apparatus (6,7) (Sirius Analytical Instruments Ltd, Forrest Row, East Sussex, UK). Ionization

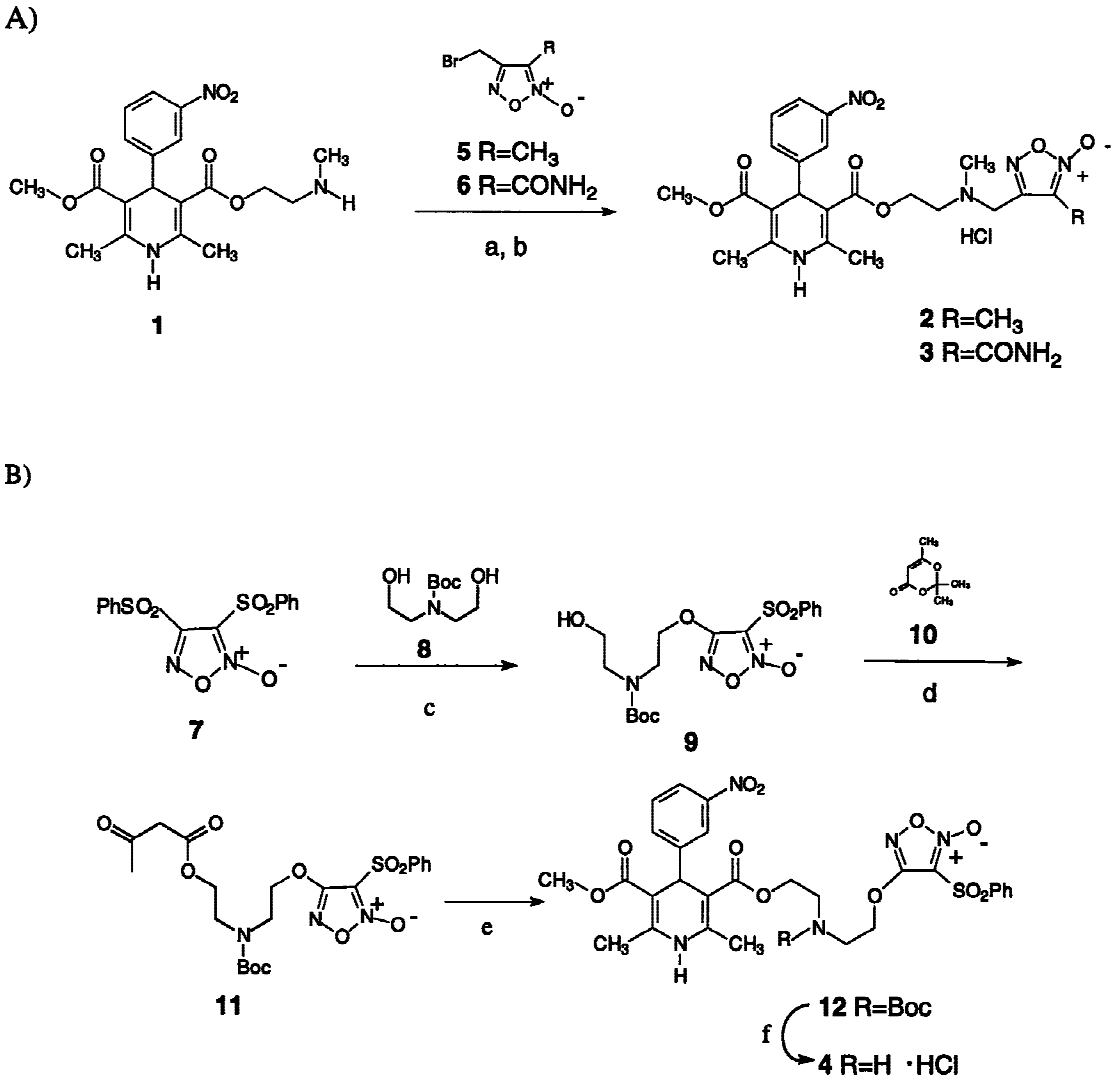

Fig. 1. Schemes illustrating the synthesis of the compounds **2**, **3** (A), **4** (B). a) **5** or **6**, $KHCO_3$ 0.5N, acetone; b) HCl saturated methanol; c) **8**, 50% NaOH, THF, –15° C; d) **10**, refluxed toluene; e) modified Hantzsch procedure: 3-nitrobenzaldehyde, methyl 3-aminocrotonate, refluxed *i*PrOH; f) HCl saturated diethyl ether.

constants were determined according to Ref. (8). The low aqueous solubility of the compounds required pK_a measurements in the presence of methanol as a cosolvent.

To obtain lipophilicity data, at least four separate titrations of ca. 0.5 mM for each compound, containing various volumes of octan-1-ol (from 0.5 ml of organic solvent/20 ml of H_2O to 13 ml of organic solvent/7 ml of H_2O), were performed in the pH range 1.8 to 9. The titrations were carried out under N_2 at 25.0 ± 0.1°C (9). Final data were obtained by the Multiset approach as described elsewhere (10) and validated by shake-flask when necessary.

RESULTS AND DISCUSSION

Chemistry

Syntheses of the 1,4-DHPs **2–4** are reported in Fig. 1. 1,4-DHPs **2** and **3** were easily obtained by stirring in acetone in the presence of potassium bicarbonate 2-(N-methylamino)ethyl methyl 2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**1**) with 4-bromomethylfuroxan **5** and **6**, respectively (Fig. 1A). Synthesis of the 1,4-DHP **4** required preliminary preparation of acetylacetic ester **11** (Fig. 1B). This intermediate was synthesized by treating 3,4-bis(phenylsulfonyl)furoxan (**7**) with N-Boc diethanolamine (**8**), in THF in the presence of 50% sodium hydroxide, at –15°C. The reaction of **9** with the synthon of acetylketene **10** in boiling toluene afforded the acetylacetate **11**. 1,4-DHP **12** was obtained by the reaction of **11** with *m*-nitrobenzaldehyde and methyl 3-aminocrotonate in refluxing isopropanol. Deprotection of the nitrogen at the lateral chain with hydrochloric acid in diethyl ether gave the expected 1,4-DHP **4**.

All the 1,4-DHPs were obtained and tested as racemic mixtures as previously described for other NO-donor 1,4-DHPs (2 and references therein).

All furoxan 1,4-DHPs discussed in the present work bear a basic nitrogen chain. The pK_as of compounds **2–4** (Table I) are quite a bit lower than the pK_a of the reference **1**. As a consequence, all the hybrid products, at physiological pH, exist largely in the neutral form, unlike the lead. Such low basicity properties are not fully explained by the presence in the lateral chain of the strong electron withdrawing furoxan moieties (11), with the only exception derivative **3**. Simple pK_a calculations confirm this assumption (12). Indeed, intramolecular effects connected with molecular flexibility could also be important in determining the basicity behavior of these compounds.

Analysis of lipophilicity data shows that, for all the investigated compounds, there is a rather good agreement between log P^N, namely the experimental lipophilicity value of the neutral form, and the corresponding CLOGP value (see Table I). This means that the lipophilicity behavior of the neutral forms is not heavily influenced by intramolecular effects, which depend on the molecular flexibility not codified in the CLOGP algorithm. All the products display quite similar log P^N values with the only exception the more lipophilic phenylsulfonyl **4**. The distribution coefficient at the physiological pH (log $D^{7.4}$) of **1**, is different from the corresponding log P^N, due to the high pK_a value of this compound, whereas the low basicity of the furoxan derivatives **2** and **3** renders their log P^N = log $D^{7.4}$.

Recently, the *diff*(log P^{N-I}), namely the difference between the lipophilicity of neutral and ionized species of a given compound, was successfully used (8) to investigate the lipophilic behavior of ionized species of compounds. As a rule, the *diff*(log P^{N-I}) covers a range from 3 to 4 in the octanol/water system. Among the *diff*(log P^{N-I}) entered in Table I, only that of **3** is in keeping. This suggests that molecular flexibility should influence the lipophilicity behavior of the remaining cations.

NO-Release

The capacity of all the final 1,4-DHPs to produce nitrite in physiological conditions, in the presence of an excess of cysteine, was evaluated by the procedure previously described (13). The results are entered in Table II. The formation of nitrite is relevant for sulfonyl derivative **4**, intermediate for carbamoyl compound **3**, and negligible for methyl derivative **2**. Detection of nitrite can be used to infer the previous presence of NO. It can be used as a measure of the original concentration of nitric oxide but only in a first ap-

Table I. Ionisation Constants and Lipophilic Descriptors in Octanol/water of the Compounds Under Study

Compound	pK_a[a]	CLOGP[b]	log P^N[c]	*diff*(log $P^{exp-calc}$)[d]	log P^I[e]	*diff*(log P^{N-I})[f]	log $D^{7.4}$[k]
1	8.78 ± 0.04	3.06	2.86 ± 0.01	–0.20	0.35 ± 0.02	2.51	1.50
2	3.83 ± 0.04	2.84	2.86 ± 0.06[g]	0.02	0.85 ± 0.09[h]	2.01	2.86
3	5.07 ± 0.02	2.18	2.85 ± 0.02	0.67	–0.08 ± 0.02[i]	2.93	2.85
4	4.59 ± 0.01	5.30	—[j]	—	—	—	—

[a] Determined by potentiometry; MeOH as cosolvent was used in percentage ranging from 30 to 65. The extrapolation to zero was obtained by the Yasuda-Shedlovsky procedure (15).
[b] Calculated log P values by CLOGP programme v. 4.0, BioByte Corp.
[c] Log P of the neutral form of the compound determined by potentiometry.
[d] Log P^N *minus* CLOGP.
[e] Log P of the cationic form of the compound determined by potentiometry otherwise specified.
[f] Log P^N – log P^I
[g] Obtained by mixed procedure: potentiometry combined with shake-flask.
[h] Validated by shake-flask.
[i] Obtained by shake-flask.
[j] Insoluble in either water or octanol.
[k] Calculated from the following equation: $D = P^N \cdot \left(\frac{1}{1+10^{pKa-pH}}\right) + P^I \cdot \left(\frac{10^{pKa-pH}}{1+10^{pKa-pH}}\right)$

Table II. Nitrite Formation and Pharmacological Results

Compound	$\%NO_2^-$ ± SE[a]	EC_{50} ± SE (nM)[b]	EC_{50}^{ODQ} ± SE (nM)[c]
Nicardipine	—	1.0 ± 0.1	—
1	—	65 ± 0.6	—
2	0.20 ± 0.11	2.5 ± 0.6	2.5 ± 0.4
3	3.8 ± 0.1	38 ± 5	35 ± 1
4	34.3 ± 1.7	30 ± 2	100 ± 10***[d]

[a] Nitrite ($\%NO_2^-$ mol/mol) was determined according to the Griess reaction in the presence of L-cysteine.
[b] Vasodilating activity.
[c] Vasodilating activity performed in the presence of 1 μM ODQ.
[d] $P < 0.0001$ when compared to EC_{50} value in the absence of ODQ; Student's *t*-test for unpaired values.

proximation because, in these conditions, NO can undergo a variety of reactions besides oxidation (14).

Pharmacology

Vasodilator activities of 1,4-DHPs were evaluated on K^+-depolarized rat thoracic aorta strips by the procedure previously described (1). EC_{50} values, calculated from concentration-response curves, are reported in Table II, as are the values in the presence of 1 μM 1H-1,2,4-oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), a well-known inhibitor of the soluble guanylate cyclase (sGC) (EC_{50}^{ODQ}). Analysis of the vasodilating properties (EC_{50}, Table II) shows that introduction of furoxan substructures into the basic 3-lateral chain affords vasodilating agents slightly more potent than the lead **1** when 3-carbamoyl (derivative **3**) and 4-phenylsulfonyl (derivative **4**) substituted furoxan moieties are used. By using the 3-methylfuroxan substructure, the rather more potent 1,4-DHP **2** is obtained. This last compound displays an activity comparable to that of nicardipine.

For compounds **2** and **3**, vasodilating potencies evaluated in the presence of 1 μM ODQ are the same as those determined in its absence (Table II, EC_{50}^{ODQ}). This means that in the vasodilator response only Ca^{2+}-channel antagonism is involved. By contrast **4**, which is a more potent NO-donor, in the presence of ODQ decreases its potency by about three times. This indicates that NO is involved in the response. No further shift of the concentration-response curve was observed on increasing the inhibitor concentration to 2 μM ODQ. It is reasonable to assume that the vasodilating activity retained in the presence of ODQ is principally due to its Ca^{2+}-channel antagonist properties (1,2). Thus, the introduction in **1** of the 3-methylfuroxan moiety and, to a lesser extent, of the 3-carbamoyl one, is beneficial for blocking the 1,4-DHP receptor, whereas the introduction of the 3-phenylsulfonylfuroxan substructure is detrimental. Analysis of the rightward shift of the concentration-response curve of **4** (Fig. 2) shows that the compound behaves as a well-balanced hybrid. In fact, in a large part of the tested concentration range, it triggers vasodilation, which depends on its NO-donor as well as its Ca^{2+}-blocker properties. The decrease in the ability to block the 1,4-DHP receptor and the increase of NO-donor properties are responsible for the balance of this hybrid with respect to unbalanced hybrids **2**, **3**.

In conclusion, these findings indicate that the use of

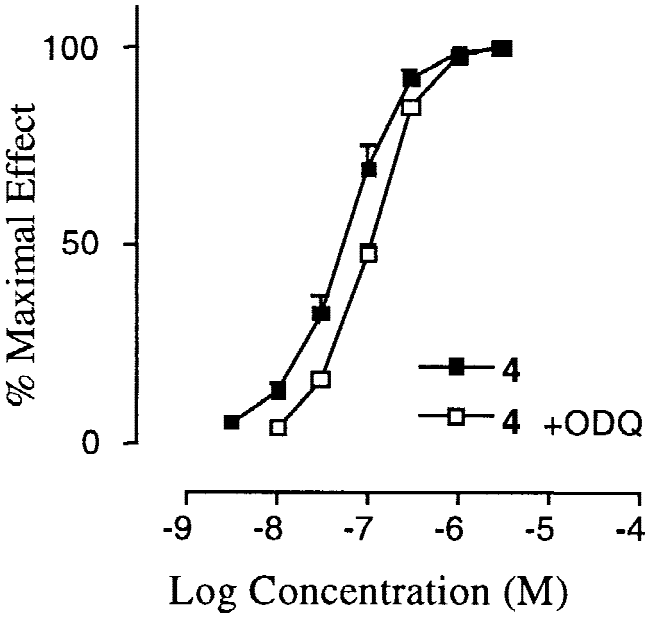

Fig. 2. Concentration-response curves for vasodilator activity of compound 4, in the presence and in the absence of ODQ. All points are mean values ± SE from independent experiments.

nicardipine analogue **1** as a reference model to build "hybrid" 1,4-DHPs endowed with mixed NO-like and Ca^{2+}-channel antagonist vasodilating properties can lead to the achievement of well-balanced hybrids when an appropriate substituted NO-donor furoxan moiety is selected. General analysis of pK_a and lipophilicity descriptors of these new DHPs suggests that molecular flexibility could influence both their basicity and log P^I.

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REFERENCES

1. A. Di Stilo, S. Visentin, C. Cena, A. M. Gasco, G. Ermondi, and A. Gasco. New 1,4-dihydropyridines conjugated to furoxanyl moieties, endowed with both nitric oxide-like and calcium channel antagonist vasodilator activities. *J. Med. Chem.* **41**:5393–5401 (1998).
2. C. Cena, S. Visentin, A. Di Stilo, D. Boschi, R. Fruttero, and A. Gasco. Studies on agents with mixed NO-dependent and calcium channel antagonist vasodilating activities. *Pharm. Res.* **18**:157–165 (2001).
3. T. Shibanuma, M. Iwanami, M. Fujimoto, T. Takenaka, and M. Murakami. Synthesis of the metabolites of 2-(N-benzyl-N-methylamino)ethyl methyl 2,6-dimethyl-4-(*m*-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride (nicardipine hydrochloride, YC-93). *Chem. Pharm. Bull.* **28**:2609–2613 (1980).
4. S. C. Bergmeier and S. L. Fundy. Synthesis of oligo(5-aminopentanoic acid)-nucleobases (APN): Potential antisense agents. *Bioorg. Med. Chem. Lett.* **7**:3155–3138 (1997).
5. G. Sorba, G. Ermondi, R. Fruttero, U. Galli, and A. Gasco. Unsymmetrically substituted furoxans. Part 16. Reaction of benzenesulfonyl substituted furoxans with ethanol and ethanethiol in basic medium. *J. Heterocyclic Chem.* **33**:327–334 (1996).
6. A. Avdeef. pH-metric log P. Part 1. Difference plots for determining ion-pair octanol-water partition coefficients of multiprotic substances. *Quant. Struct.-Act. Relat.* **11**:510–517 (1992).
7. G. Caron, P. Gaillard, P. A. Carrupt, and B. Testa. Lipophilicity

behavior of model and medicinal compounds containing a sulfide, sulfoxide, or sulfone moiety. *Helv. Chim. Acta* **80**:449–462 (1997).
8. R. Fruttero, G. Caron, E. Fornatto, D. Boschi, G. Ermondi, A. Gasco, P. A. Carrupt, and B. Testa. Mechanisms of liposomes/water partitioning of (p-methylbenzyl)alkylamines. *Pharm. Res.* **15**:1407–1413 (1998).
9. A. Avdeef. Assessment of distribution-pH profiles. In V. Pliska, B. Testa, and H. van de Waterbeemd (eds.), *Lipophilicity in Drug Action and Toxicology*, VCH Publishers, Weinheim, 1996 pp. 109–139.
10. A. Avdeef, K. J. Box, J. E. A. Comer, C. Hibbert, and K. Y. Tam. pH-metric log P. 10. Determination of vesicle membrane-water partition coefficients of ionizable drugs. *Pharm. Res.* **15**:209–215 (1998).
11. R. Fruttero, D. Boschi, E. Fornatto, A. Serafino, A. Gasco, and O. Exner. Electronic substituent effects of furoxan and furazan system. *J. Chem. Res.* (*S*): 495–496 (1998).
12. D. D. Perrin, B. Dempsey, and E. P. Serjeant. *pK_a Prediction for Organic Acids and Bases*, Chapman and Hall, London, 1981; PALLAS for Windows, v. 1.3, *pK_a Prediction Module*: pKalc 3.2, 1995.
13. C. Medana, A. Di Stilo, S. Visentin, R. Fruttero, A. Gasco, D. Ghigo, and A. Bosia. NO donor and biological properties of different benzofuroxans. *Pharm. Res.* **16**:956–960 (1999).
14. M. Feelisch and J. S. Stamler (eds.). *Methods in Nitric Oxide Research*, John Wiley and Sons, Chichester, UK, 1996.
15. A. Avdeef, J. E. A. Comer, and S. J. Thomson. pH-Metric log P. 3. Glass electrode calibration in methanol-water, applied to pK_a determination of water-insoluble substances. *Anal. Chem.* **65**:42–49 (1993).