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Solvation enthalpies as descriptors of structure – in vitro percutaneous permeation relationship of benzoxazinones regioisomers

Paola Minghetti [a,]*, Antonella Casiraghi [a], Francesco Cilurzo [a], Luisa Montanari [a], M. Valmen Monzani [b], Giorgio Bertolini [b], Andrea Zaliani [b]

[a] *Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Milano, Viale Abruzzi 42, I-20131 Milan, Italy*
[b] *Italfarmaco Research Centre, Via dei Lavoratori 54, I-20092 Cinisello B. (MI), Italy*

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Dedicated to Professor Pietro Pratesi

Abstract

The aim of this work was to correlate the in vitro human skin permeability, expressed as the permeability coefficient (K_p), and some physicochemical parameters of a new series of benzoxazinones. The in vitro human skin permeability of 14 substances, including regioisomers with CH_3, OH, OCH_3, and Cl groups in different positions on the aromatic ring, was determined. The modified Franz diffusion cell method was used. The K_p values were in the range 0.14–8.24 cm/h, showing a strong dependence on the position and type of substituent. Physicochemical descriptors usually referred in literature, such as log P, molecular weight and volume (MV), hydrogen bond donor (H_d) and acceptor activity (H_a), and molecular refractivity were considered, with the addition of solvation enthalpy ($\Delta\Delta H_{solv}$). $\Delta\Delta H_{solv}$ is defined as the difference between formation enthalpies in water and octanol. The algorithm with the best correlation between K_p and physicochemical descriptors was calculated, taking into account the differences observed among the regioisomers. The algorithm obtained with $\Delta\Delta H_{solv}$ had a good correlation ($r^2 = 0.749$, $F = 16.43$, $P = 0.0005$), comparable with the equation, proposed by Potts and Guy, based on MV, H_d and H_a ($r^2 = 0.830$, $F = 16.3$, $P = 0.0004$). © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Benzoxazinones; In vitro percutaneous absorption; Regioisomer; Physicochemical characteristics; Structure–property relationship

1. Introduction

Organic nitrates and nitroglycerin have been mainstays of cardiovascular therapies for many years. The main drawbacks of these molecules include either pharmacokinetic deficiencies such as poor absorption in the GI tract and an extensive first-pass hepatic metabolism or side effects associated with the action on the arteriolar vessels leading to marked hypotension.

A new original nitrates class has been developed with the aim to have only specific nitric oxide-dependent action and a preferential effect on large conductance arterial vessels. These compounds showed a reduced hypotensive action together with a marked antiischemic activity evaluated as the protective action against vasopressin-induced ECG changes in rats. Clinical trials confirmed for the lead compound of this class, sinitrodil, the marked antianginal activity and the preferential action on large coronary vessels [1].

Considering the transdermal route suitable for the administration of these drugs, the ability of the sinitrodil to reach therapeutical steady-state plasma concentrations was evaluated in a previous work obtaining good results [2].

A series of benzoxazinones analogues of sinitrodil was synthesised [3]. We selected 14 substances of this series to study in vitro human skin permeability (Fig. 1).

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* Corresponding author. Tel.: +39-02-294 05002; fax: +39-02-295 14197.
E-mail address: paola.minghetti@unimi.it (P. Minghetti).

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The aim of this work was to find a correlation between in vitro human skin permeability and some physicochemical parameters of the selected substances, differing in both substituent groups and their positions on the aromatic ring. The peculiarity of the studied series was the possibility of evaluating the influence of different regioisomers with the same substituents (CH_3, OH, OCH_3, Cl) in different positions.

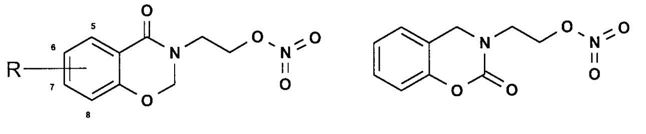
In recent years several models have been developed to predict the transport of the molecules through human skin. Log *P*, a measure of lipophilicity, and molecular size are the two basic physicochemical parameters that determine the transport.

The equation proposed by Potts and Guy [4] to examine a number of different sets of percutaneous permeability data is as follows:

$$\text{Log}\,K_p = -6.3 + 0.71\log P - 0.0061\text{MW} \qquad (1)$$

where K_p is the permeability coefficient, $\log P$ is the partition coefficient in octanol/pH 7.4 phosphate-buffered saline solution and MW is the molecular weight. The permeability results are affected primarily by the lipophylic character of a drug and are inversely dependent upon molecular weight. This is a simple model able to predict skin permeability.

The relevance of lipophilicity and molecular size is not always enough effective to predict the permeability coefficient of drugs through skin, so further descriptors have been introduced. The target was to improve description of permeability and to consider the molecular details of drug–lipid interactions during transport through the stratum corneum. For this reason the organic–water partition coefficient has been more explicitly represented introducing the terms of dipolarity/polarizability, hydrogen bond donor and acceptor activity, and molar refractivity [5].



1a & c-p 1b

Compounds	R
Sinitrodil (1a)	H
1b	H
1c	5-Methyl
1d	6-Methyl
1e	6-Hydroxy
1f	6-Methoxy
1g	6-Chloro
1h	6-Nitro
1i	7-Methyl
1l	7-Hydroxy
1m	7-Methoxy
1n	7-Chloro
1o	7-Trifluoromethyl
1p	8-Methyl

Fig. 1. Compounds list and substituents.

Potts and Guy [5] obtained a second equation that allows prediction of permeability based on permeant's size and hydrogen bonding activity:

$$\text{Log}\,K_p = -4.85 + 0.026\text{MV} - 1.72H_d - 3.93H_a \qquad (2)$$

where K_p is the permeability coefficient, MV is the molecular volume, H_d and H_a are hydrogen bond donor and acceptor activity, respectively.

It is interesting to note that the molecular volume coefficient is positive. This is probably because MV represents a combination of physical phenomena, i.e. the impact of molecular size on partitioning and diffusion.

In this work we verified how some of the algorithms found in literature fitted our data. Furthermore other parameters, such as molecular refractivity and solvation enthalpies, were introduced in order to find an algorithm that could also consider differences strictly related to the structure of the molecules.

2. Experimental

2.1. Materials

Compounds **1a**–**p** (Fig. 1) were synthesised at the Italfarmaco Research Centre [3]. All substances and other chemicals were used as obtained.

2.2. Methods

2.2.1. Determination of drug solubility (S)

The solubility of each drug in water:polyethylene glycol 400 (80:20 v/v) was obtained by equilibrating large excess of the solute and vehicle for 24 h. Each solution was stirred vigorously throughout the experiment and the temperature was maintained at 32 ± 1°C. After equilibration, an aliquot of sample was filtered quickly with a membrane filter (pore size 0.22 μm, Millex®-GV Millipore). When the solution was appropriately diluted with methanol, the concentration was determined by HPLC, with the method described below.

2.2.2. Determination of partition coefficient (P)

The partition coefficient of each compound was determined in octanol/pH 7.4 phosphate-buffered saline solution (PBS, Ph. Eur., ed. 1997, p. 274). The shake

Table 1
HPLC conditions for the analysis of drugs

Drug	Mobile phase (v/v)	Flow rate (ml/min)	Wavelength (nm)
Sinitrodil (**1a**)	methanol:water:tetrahydrofuran (54:43:3)	1.2	235
1b	methanol:water:tetrahydrofuran (54:43:3)	1.0	220
1c	methanol:water:tetrahydrofuran (54:43:3)	1.2	247
1d	methanol:water:tetrahydrofuran (54:43:3)	1.2	235
1e	methanol:water (40:60)	1.2	220
1f	methanol:water:tetrahydrofuran (54:43:3)	1.0	220
1g	methanol:water:tetrahydrofuran (54:43:3)	1.0	220
1h	methanol:water:tetrahydrofuran (54:43:3)	1.2	235
1I	methanol:water:tetrahydrofuran (54:43:3)	1.2	247
1l	methanol:water (40:60)	1.2	220
1m	methanol:water:tetrahydrofuran (54:43:3)	1.2	264
1n	methanol:water:tetrahydrofuran (54:43:3)	1.2	247
1o	methanol:water:tetrahydrofuran (54:43:3)	1.2	235
1p	methanol:water:tetrahydrofuran (54:43:3)	1.2	247

flask method was used. After vigorous mixing for 2 h at room temperature (r.t.) the phases were centrifuged, appropriately diluted and the aqueous phase was assayed by HPLC, with the method described below.

2.2.3. In vitro permeation studies

Human abdomen skin was used for the skin permeation studies. Skin samples, obtained by surgical operation, were harvested from the abdomen, then immediately refrigerated at −20°C. Frozen skin samples were thawed within 48 h of removal and the epidermal layer (comprising the stratum corneum and viable epidermis) were separated by immersing the skin in distilled water at 60 ± 1°C for 1 min and peeling it from the derma. The heat-separated stratum corneum and epidermis (SCE) membranes were dried in a desiccator at approximately 25% RH, wrapped in aluminium foil and stored at 4 ± 1°C until use. Dried SCE samples were rehydrated at r.t. by immersion in saline solution for about 16 h before use. Each membrane was carefully mounted on a modified Franz-type diffusion cell of approximately 5 ml receiver capacity and fastened with a rigid clamp. These cells, respect to the original Franz-type diffusion cell, had a wider vertical column and the bowl shape was removed. These cells had a diffusion area of 0.636 cm^2. Each cell was individually calibrated with respect to its receiver volume and diffusion area.

At the start of the experiment, 1 ml of water:polyethylene glycol 400 (80:20 v/v) solution containing a saturated solution with large excess of the same drugs was applied to the diffusion cell as donor phase.

The receiver medium was constituted with 0.9% NaCl solution:polyehtylene glycol 400 (80:20 v/v) solution containing 100 μg/ml streptomycin (Sigma Chemical Co., USA) as preservative. The receiver medium was continuously stirred with a small magnetic bar and thermostated at 37 ± 1°C, so that the skin surface temperature was 32 ± 1°C. At predetermined times (0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h) 0.2 ml samples were withdrawn from the receiver compartment and replaced with fresh receiver medium. Sink conditions were maintained throughout the experiments. Samples were analysed by HPLC, with the method described below. All the experiments were run in triplicate.

2.2.4. Drug assay

The concentrations of each compound were determined by HPLC assay (HP 1100, Chemstations, Hewlett Packard, USA).

Samples (20 μl) were injected at r.t. on a reverse-phase column (C18, 5 μm Spherisorb ODS2, 20 cm-Waters HPLC). The chromatographic conditions were adjusted in order to obtain the best separation for each compound and are reported in Table 1. All the mobile phases were filtered and degassed before using. The standard curves were obtained by plotting the peak area as a function of drug concentration of six known concentrations of substances ranging from 1 to 50 μg/ml and prepared using HPLC-grade methanol. Calibration curves demonstrated linearity over the concentration range encountered in the samples.

2.2.5. Data analysis

The cumulative amount permeated through the SCE per unit area was calculated from the concentration of each substance in the receiving medium and plotted as a function of time. Each data point on the plot represents a mean of triplicate permeation experiments. The flux (J) was determined as the slope of the linear portion of the plot.

The permeability coefficient was calculated following the Fick's first law of diffusion:

$$K_p = J_{max}/S$$

Table 2
Physicochemical properties and permeability parameters of the tested compounds [a]

Comp.	MW (Da)	M.p. (°C)	S (µg/ml)	Log P		J (µg/cm^2 per h)	$K_p \times 10^3$ (cm/h)
				Calculated	Experimental		
Sinitrodil (**1a**)	238.2	55.5–56.5	2604 ± 18	1.85	1.86 ± 0.02	9.91 ± 1.50	3.81 ± 0.58
1b	238.2	103	500 ± 15	1.85	1.82 ± 0.13	1.64 ± 0.75	0.71 ± 0.33
1c	252.2	72–74	568 ± 8	2.41	2.54 ± 0.01	2.58 ± 0.09	4.54 ± 0.16
1d	252.2	77–78	669 ± 5	2.41	2.41 ± 0.05	3.86 ± 0.76	5.78 ± 1.09
1e	254.2	80–82	3477 ± 23	1.45	1.63 ± 0.00	1.05 ± 0.33	0.30 ± 0.09
1f	268.2	62	1490 ± 128	1.83	2.07 ± 0.08	5.49 ± 1.66	3.68 ± 1.11
1g	272.6	98–99	134 ± 1	2.56	2.30 ± 0.13	0.90 ± 0.34	6.73 ± 2.52
1h	283.2	100–101	106 ± 1	1.57	1.66 ± 0.14	0.37 ± 0.048	3.54 ± 0.45
1I	252.2	90–92	352 ± 13	2.41	2.44 ± 0.03	2.90 ± 0.04	8.24 ± 0.11
1l	254.2	131–133	2306 ± 3	1.45	1.63 ± 0.01	0.31 ± 0.09	0.14 ± 0.04
1m	268.2	101–102	227 ± 2	1.83	2.09 ± 0.00	0.56 ± 0.14	2.49 ± 0.60
1n	272.6	86–88	226 ± 1	2.56	2.74 ± 0.03	1.62 ± 0.26	7.17 ± 1.17
1o	306.2	50–51	167 ± 11	2.73	3.01 ± 0.02	1.27 ± 0.07	7.63 ± 0.41
1p	252.2	75–76	615 ± 30	2.41	2.48 ± 0.00	2.15 ± 0.31	3.50 ± 0.51

[a] MW, molecular weight; M.p., melting point; S, solubility; log P, partition coefficient; J, flux; K_p, permeability coefficient.

where K_p is the permeability coefficient (cm/h), J_{max} is the maximum flux (µg/cm^2 per h) and S is the drug donor concentration (µg/ml), corresponding to the drug solubility in the vehicle.

2.2.6. Computational and statistical data analysis

Calculated log P values were obtained within the modelling software SYBYL 6.2 (Tripos Inc., St. Louis, MO) using the Rekker's algorithm [6].

All the molecular structures have been built and edited using the program CERIUS2 (Molecular Simulations Inc., San Diego, CA) and set in their lowest energy conformations according to the crystal structure of sinitrodil (**1a**).

A semiempirical calculation was then applied to each of the molecules within AM1 Hamiltonian using the solvation implementation of Dixon et al. [7]. Gas phase formation enthalpies (ΔH_{gp}) and formation enthalpies in water (ΔH_{wa}) and in octanol (ΔH_{oct}) were then calculated. The aqueous solvation enthalpies $\Delta H_{wsol} = \Delta H_{gp} - \Delta H_{wa}$ and the octanol solvation enthalpies $\Delta H_{octsol} = \Delta H_{gp} - \Delta H_{oct}$ for each compound were used to calculate the solvation enthalpy, $\Delta\Delta H_{solv} = \Delta H_{wsol} - \Delta H_{octsol}$. This entity was used for the multilinear regression analysis. The BMDP 1.0 (BMDP Statistical Software Inc., USA) statistical package was used for this analysis. Molecular volumes were calculated using CERIUS2.

3. Results and discussion

Some relevant physicochemical characteristics of the tested compounds are reported in Tables 2 and 3. Calculated octanol–water partition coefficients of neutral compounds are frequently taken as best approximation of experimental values. Table 2 shows that this is the case for our molecules except for the halogenated ones (**1g**, **1n** and **1o**) and for oxygenated derivatives (**1e**, **1f**, **1l** and **1m**) where a difference of about 10% of the calculated log P from experimental one can be appreciated. This could be likely due to the lack of good parameters for halogens, which often do not account for their H-bond forming propensity, and to the salicylate moiety which often deserves ambiguity in incremental-based algorithms, respectively. Thus, benzoic acid or phenol taken as template may diverge in a incremental substituent effect. Another important issue is that concerning log P calculation of aromatic heterocycles, as in the case of benzoxazinones, since it has been optimised for aromatic polycarbons. This applica-

Table 3
Physicochemical properties parameters of the tested compounds [a]

Comp.	MV (Å^3)	HB$_a$	HB$_d$	$\Delta\Delta H_{solv}$ (kcal/mol)
Sinitrodil (**1a**)	241.04	5	0	−10.78
1b	247.06	5	0	−11.23
1c	266.02	5	0	−12.92
1d	267.42	5	0	−11.80
1e	257.79	6	1	−15.70
1f	278.61	6	0	−14.07
1g	265.11	5	0	−12.74
1h	277.53	7	0	−4.42
1I	267.42	5	0	−11.69
1l	257.72	6	1	−15.71
1m	278.59	6	0	−13.70
1n	265.11	5	0	−11.67
1o	287.54	5	0	−12.06
1p	267.03	5	0	−11.65

[a] MV, molecular volume; HB$_a$, hydrogen-bond acceptor site count; HB$_d$, hydrogen-bond donor site count; MR, molecular refractivity; $\Delta\Delta H_{solv}$, solvation enthalpy.

tion is not always acceptable and corrections have been suggested for a limited number of aromatic heterocycles [8].

The permeation was constant for each molecule during all the selected time ($r^2 > 0.992$). Fluxes and K_p of molecules with the same substituent in different positions were statistically different ($P < 0.01$) among them (Table 2).

Sinitrodil (**1a**), that has no substituent in the benzoxazinone system, showed the highest flux with a lag time practically equal to zero. All the other compounds showed smaller fluxes compared with that of sinitrodil (**1a**), but the introduction of CH_3, Cl and CF_3 increased in a very significant way the K_p especially when the functional group was introduced in position 7 (Table 2).

The chemical differences for the heterocycles (lactame and carbamate group) in **1a** and **1b**, without aromatic substituents, did not have any influence to $\log P$, but only to the melting point and solubility. These two compounds showed a marked difference in J and K_p; these results are difficult to explain only using Eq. (1).

The substances substituted in the 6 position (**1d**, **1e**, **1f**, **1g**) of the benzoxazinone showed fluxes higher than those of the molecules synthesised with the same substituent in position 7, besides the substances **1g** and **1n** that showed quite similar fluxes. This behaviour can not be explained completely by using these experimental parameters. Hydroxylic groups increase the solubility of the molecules but greatly decrease the coefficient of permeability.

The influence of the methyl group in all positions of the aromatic ring of the benzoxazinone system was also investigated. The four molecules (**1c**, **1d**, **1i**, **1p**) showed similar $\log P$, melting points and solubilities with the exception of **1i** that has a higher melting point and lower solubility. The four J or K_p values were significantly different ($P < 0.01$) and **1i** had the highest value. Also in this case, in order to explain the skin permeability of the tested set of compounds, it is necessary to introduce other description variables.

Potts and Guy reported [4] a good correlation between $\log P$, molecular weight and K_p (as reported in Eq. (1)). However, using this literature approach we were unable to obtain statistically sound correlations with experimental data ($r^2 = 0.547$, $F = 6.65$, $P < 0.02$). On the contrary, the more chemically accurate Eq. (2) gave us good results using $\log K_p$ ($r^2 = 0.830$, $F = 16.31$, $P = 0.0004$). This fact prompted us to better describe the molecular set in terms of more sophisticated and structure-derived measurements [9].

As water desolvation could be an important parameter for the permeability equilibria, we thought that enthalpy associated with a phase transition (vacuum toward solvated medium) for the molecule could be an effective descriptor. Formation enthalpy in vacuo can be easily and accurately accessed by means of semiempirical calculations while the same entity in the condensed phase was a more challenging target. Considerable efforts have been done in recent years to set up reliable models of solvation energy calculation which did not need huge amount of explicit solvating molecules and which could be easily and routinely accomplished by computational chemistry software. Among others, the continuum solvation models proved to be particularly accurate in predicting formation enthalpies in solution [7]. Taking advantage from the octanol and water parameterisation of AM1 Hamiltonian, we calculated the enthalpies of formation for both solvents and in vacuo for each molecule. Linearly combining the results in a thermodynamic cycle we defined solvation enthalpy ($\Delta\Delta H_{solv}$) as the energy gained or lost by the molecule changing its phase from a solvent to another. These calculated parameters are relative and can be thus used as physicochemical descriptors of different molecular behaviours as solutes. They turned out to be effective in fitting, allowing to predict $\log K_p$ using both $\log P$ and $\Delta\Delta H_{solv}$:

$$\mathrm{Log}\, K_p = -3.57 + 5.04 \log P + 0.27 \Delta\Delta H_{solv}$$

$$r^2 = 0.749,\ F = 16.43,\ P = 0.0005.$$

$\Delta\Delta H_{solv}$ works as a sort of correction of $\log P$. Calculated $\Delta\Delta H_{solv}$ are always negative indicating a gain in formation enthalpies both in water and in octanol. Negative $\Delta\Delta H_{solv}$ showed a higher gain in enthalpies of formation in water than in octanol and hence, in the observed sample series and just relating to it, more hydrophilic compounds have more negative $\Delta\Delta H_{solv}$. This fact suggests a deep connection between the forces, which direct solvation and those, which manage permeation through a likely desolvation step. In this respect $\Delta\Delta H_{solv}$ could be assimilated to $\Delta \log P$ [10] which has been used for the same purpose and was believed to describe hydrogen-bond capacity. Surprisingly the 6- and 7-hydroxy derivatives (**1e** and **1l**) turned out to be much less hydrophilic than expected. In this case $\log P$ (1.63 for both) and $\Delta\Delta H_{solv}$ could be used to explain their different permeability coefficients respect to nitro aromatic derivative ($\log P = 1.66$, $\Delta\Delta H_{solv} = -4.42$). In particular the lowest value of **1l** could be likely explained by the different acidity of the hydroxy group in para to carbonyl. This finding underlines the importance of benzoxazinone carbonyl group as a primary site of solvation together with the influencing role of substituent position and type.

The correlation coefficient of the second equation of Potts and Guy resulted slightly better than that obtained using $\log P$ and $\Delta\Delta H_{solv}$.

Nevertheless, this new algorithm should be investigated by using a larger molecules set as both the selected variables showed a significativity ($\log P$: $P =$

0.0002; $\Delta\Delta H_{\mathrm{solv}}$: $P = 0.012$) at least comparable with that of the parameters used in the second Potts and Guy equation (MV: $P = 0.063$; H_{d}: $P = 0.001$; H_{a}: $P = 0.141$).

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