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# Interaction of monoamine oxidase inhibitory drugs with some phospholipids

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**Abstract**: The effect of 17 monoamine oxidase inhibitory drugs (propargylamine derivatives) on the phase transition parameters of some synthetic phospholipids has been studied by differential scanning calorimetry. The drugs modified the thermal behaviour of phospholipids proving the existence of phospholipid–drug interactions. Phosphatidylethanolamine, phosphatidylcholine and phosphatidic acid equally interact with the monoamine oxidase inhibitory drugs. Significant correlations were found between the change of the phase transition parameters of phospholipids and the hydrophilic and hydrophobic molecular parameters of drugs. Calculations suggest that both hydrophilic (between the head group of phospholipids and the polar propargylamine group of drugs) and hydrophobic (between the apolar fatty acid chains of phospholipids and the lipophilic substructures of drugs) forces are involved in the phospholipid–drug interaction. Chloro substitution in the hydrophobic moiety of drugs enhances considerably the strength of interaction.

**Keywords**: *Monoamine oxidase inhibitors; interaction with phospholipids; differential scanning calorimetry.*

## Introduction

Differential scanning calorimetry (DSC) has become a standard technique for studying the thermal induced transition of phospholipid bilayers from an ordered, crystalline-like state at low temperature (gel phase) to a liquid crystalline-like state at higher temperature. The change of main transition temperature and peak width (related to molecular cooperativity) indicate the structural changes caused by the insertion of interacting molecules into the lipid bilayer. The application of calorimetry in the study of properties of biological and lipid model membranes and the theory and practice of DSC measurements have been recently reviewed [1, 2]. The changes of the transition parameters in the presence of bioactive compounds are indicative of the interactions of these compounds with the membranes of the target organisms [3, 4]. The correlation between structural and functional changes is similar in the lipid bilayers of biological and model membranes [5]. Therefore, the hydrated phospholipids are a suitable model system to study the effects of membrane modifying agents [6–10].

Propargylamine derivatives are selective inhibitors of B-type monoamine oxidase [11, 12]. According to our knowledge the exact mode of action of these drugs has not been elucidated in detail. The objectives of our investigations were the study of the interaction of some monoamine oxidase (MAO) inhibitory drugs with phospholipids and to correlate the membrane damaging effect with their physicochemical parameters.

## Experimental

Dimirystoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylethanolamine (DPPE) and dipalmitoylphosphatidic acid (DPPA) were purchased from Sigma Co. (St Louis, MO) and were applied without additional purification. The chemical structure of monoamine oxidase inhibitory drugs are shown in Table 1. Phospholipids and the MAO inhibitory drugs were dissolved in chloroform in the molar ratio of 10:1 and then the solvent was evaporated in a nitrogen atmosphere at room temperature. Then doubly-distilled water was added to the sample in a weight ratio of water–phospholipid (4:1, v/v). The samples were vigorously mixed for 30 min above the phase transition temperature of phospholipid–drug mixtures in a Vortex mixer. The measurements were carried out on a Du Pont Thermoanalyser

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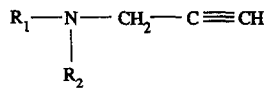
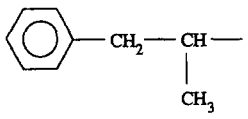
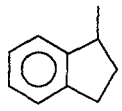
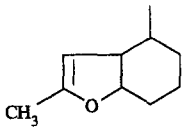
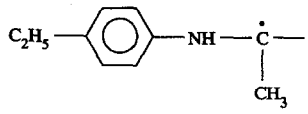
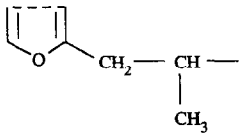
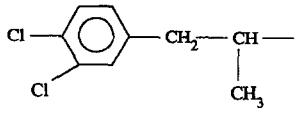
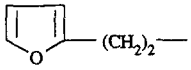
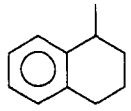
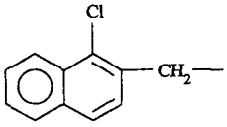
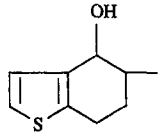
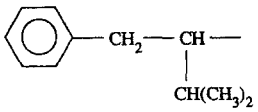
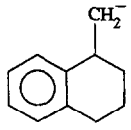
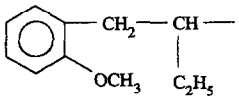
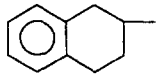
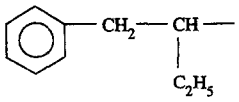
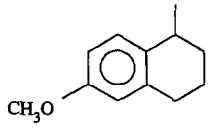
**Table 1**
Chemical structure of monoamine oxidase inhibitors



General structure

| Compound no. | $R_1$ | $R_2$ | Compound no. | $R_1$ | $R_2$ |
|---|---|---|---|---|---|
| 1 (+)<br>2 (−) |  | $-CH_3$<br>$-CH_3$ | 10 |  | $-CH_3$ |
| 3 |  | $-CH_3$ | 11 |  | $-H$ |
| 4 |  | $-H$ | 12 |  | $-CH_3$ |
| 5 |  | $-CH_3$ | 13 |  | $-CH_3$ |
| 6 |  | $-CH_3$ | 14 |  | $-CH_3$ |
| 7 |  | $-CH_3$ | 15 |  | $-CH_3$ |
| 8 |  | $-CH_3$ | 16 |  | $-CH_3$ |
| 9 |  | $-C_4H_7$ | 17 |  | $-CH_3$ |

990 (Barley Mill, Wilmington, DE) at a heating rate of 5°C $min^{-1}$ and in the sensitivity range of 0.1–0.2 mw $cm^{-1}$. The equipment was calibrated using indium. The main transition temperature ($T_m$) and the half width of main transition ($\Delta T_{1/2}$) related to the cooperativity were calculated. To study the effect of ionic environment on the phospholipid–drug interaction, the measurements with DMPC were also carried out in the presence of 0.2 M NaCl.

Stepwise regression analysis was applied to find the physicochemical parameters of drugs accounting for their interaction with phospholipids [13]. The main transition temperatures and the peak half widths of DMPC, DMPC + NaCl, DPPE and DPPA (eight altogether) were the dependent variables. The independent variables were in each case the 14 physicochemical parameters of MAO inhibitory drugs taken from ref. 14. The hydrophobic and hydrophilic molecular characteristics of monoamine oxidase inhibitory drugs were determined by means of various chromatographic techniques. The acceptance

level for the individual independent variables was set to 95% significance level. In the common multivariate regression analysis the inclusion of not significant variables in the equation lessens the significance level of the other variables. Stepwise regression analysis eliminates the not significant independent variables from the selected equation enhancing in this manner the information power of the calculation.

To study the similarities and dissimilarities between the phase-transition parameters and MAO inhibitory drugs principal component analysis (PCA) was applied [15]. The eight phase transition parameters and the 17 drugs were the variables and observations, the calculation was carried out on the correlation matrix. The PC loadings and variables were visualized by the nonlinear mapping technique [16].

## Results and Discussion

The phase transition parameters of phospho-

**Table 2**
Main transition temperature ($T_m$: °C) and peak half width ($\Delta T_{1/2}$: °C) of various DMPC–drug mixtures in distilled water (A) and in 0.2 M NaCl (B). DMPC:drug molar ratio 10:1. Numbers refer to monoamine oxidase inhibitory drugs in Table 1

| | A | | B | |
|---|---|---|---|---|
| Drug no. | $T_m$ | $\Delta T_{1/2}$ | $T_m$ | $\Delta T_{1/2}$ |
| Control | 23.8 ± 0.2 | 1.0 ± 0.1 | 24.0 ± 0.2 | 1.2 ± 0.1 |
| 1 | 23.0 ± 0.2 | 1.2 ± >0.1 | 21.5 ± 0.1 | 1.9 ± 0.1 |
| 2 | 23.2 ± 0.2 | 1.3 ± 0.1 | 21.6 ± >0.1 | 2.0 ± >0.1 |
| 3 | 22.0 ± >0.1 | 1.3 ± 0.1 | 20.6 ± 0.2 | 2.5 ± 0.1 |
| 4 | 23.0 ± 0.1 | 1.2 ± 0.1 | 23.6 ± 0.2 | 1.8 ± 0.1 |
| 5 | 22.8 ± >0.1 | 1.2 ± >0.1 | 22.4 ± 0.1 | 2.2 ± 0.1 |
| 6 | 21.8 ± 0.2 | 4.5 ± 0.5 | 24.0 ± 0.1 | 3.6 ± 0.1 |
| 7 | 21.9 ± 0.2 | 1.8 ± 0.2 | 19.9 ± 0.2 | 2.4 ± 0.1 |
| 8 | 21.8 ± 0.2 | 1.8 ± 0.2 | 19.6 ± 0.2 | 2.2 ± 0.1 |
| 9 | 21.9 ± 0.1 | 2.2 ± 0.2 | 21.8 ± 0.1 | 2.4 ± 0.3 |
| 10 | 22.0 ± >0.1 | 1.9 ± 0.1 | 22.1 ± 0.2 | 2.1 ± 0.2 |
| 11 | 22.5 ± >0.1 | 1.3 ± >0.1 | 23.1 ± 0.1 | 1.8 ± >0.1 |
| 12 | 19.6 ± 0.2 | 2.2 ± 0.2 | 23.8 ± 0.1 | 2.5 ± 0.2 |
| 13 | 22.7 ± >0.1 | 1.5 ± >0.1 | 22.9 ± 0.2 | 2.1 ± 0.1 |
| 14 | 22.9 ± 0.1 | 1.8 ± 0.1 | 22.1 ± 0.1 | 1.9 ± 0.2 |
| 15 | 21.9 ± 0.1 | 2.0 ± >0.1 | 20.6 ± 0.2 | 2.4 ± 0.2 |
| 16 | 21.8 ± 0.2 | 1.9 ± 0.1 | 20.4 ± 0.1 | 2.3 ± 0.1 |
| 17 | 22.9 ± 0.1 | 1.9 ± 0.1 | 22.0 ± >0.1 | 2.4 ± 0.1 |

**Table 3**
Main transition temperature ($T_m$: °C) and peak half width ($\Delta T_{1/2}$: °C) of DPPE–drug and DPPA–drug mixtures. Phospholipid:drug molar ratio 10:1. Numbers refer to monoamine oxidase inhibitory drugs in Table 1

| | DPPE | | DPPA | |
|---|---|---|---|---|
| Drug no. | $T_m$ | $\Delta T_{1/2}$ | $T_m$ | $\Delta T_{1/2}$ |
| Control | 65.0 ± 0.5 | 1.5 ± 0.2 | 63.5 ± 0.2 | 2.2 ± 0.1 |
| 1 | 64.3 ± 0.3 | 1.8 ± 0.1 | 63.5 ± >0.1 | 2.2 ± 0.2 |
| 2 | 64.0 ± 0.2 | 2.0 ± >0.1 | 63.4 ± 0.2 | 2.3 ± 0.1 |
| 3 | 64.3 ± 0.1 | 2.0 ± >0.1 | 63.5 ± 0.3 | 3.0 ± >0.1 |
| 4 | 64.6 ± 0.2 | 1.6 ± 0.1 | 63.5 ± >0.1 | 2.3 ± >0.1 |
| 5 | 64.5 ± >0.1 | 1.5 ± 0.1 | 63.3 ± >0.1 | 2.2 ± >0.1 |
| 6 | 62.7 ± 0.3 | 2.8 ± 0.2 | 63.7 ± 0.1 | 2.2 ± >0.1 |
| 7 | 64.1 ± >0.1 | 2.0 ± >0.1 | 63.7 ± >0.1 | 2.4 ± 0.1 |
| 8 | 64.5 ± 0.2 | 2.2 ± 0.2 | 63.5 ± 0.2 | 2.2 ± 0.1 |
| 9 | 63.2 ± 0.2 | 3.1 ± 0.1 | 63.7 ± 0.2 | 2.3 ± >0.1 |
| 10 | 64.1 ± 0.1 | 1.8 ± 0.1 | 63.5 ± >0.1 | 2.3 ± >0.1 |
| 11 | 63.0 ± >0.1 | 2.8 ± 0.3 | 62.5 ± 0.3 | 2.6 ± 0.5 |
| 12 | 61.0 ± 0.3 | 3.8 ± 0.2 | 60.5 ± 0.2 | 2.7 ± 0.2 |
| 13 | 64.1 ± 0.3 | 2.0 ± 0.3 | 62.5 ± 0.2 | 2.5 ± 0.1 |
| 14 | 63.9 ± 0.2 | 2.1 ± 0.2 | 62.0 ± 0.2 | 2.6 ± 0.1 |
| 15 | 63.0 ± >0.1 | 2.5 ± >0.1 | 62.5 ± 0.1 | 2.4 ± 0.1 |
| 16 | 62.9 ± 0.1 | 2.8 ± 0.1 | 62.5 ± >0.1 | 2.3 ± 0.2 |
| 17 | 63.8 ± 0.2 | 2.4 ± 0.2 | 62.8 ± 0.2 | 2.3 ± 0.1 |

lipid–drug mixtures are compiled in Tables 2 and 3. The drugs generally decrease the phase transition temperature of phospholipids and cause peak broadening. This finding suggests that the hydrophobic moiety of the drugs penetrates into the apolar fatty acid chains causing disordered membrane structure. However, the data do not exclude the possibility of hydrophilic interactions between the head group of phospholipids and the polar propargylamine substructure in the drugs. The fact that in an ionic environment the effect of drugs on the phase transition parameters of DMPC is different lends support to the hypothesis that both hydrophilic and hydrophobic forces are involved in the phospholipid–drug interactions. The parameters of the relationships between the membrane modifying effect of drugs and their physicochemical characteristics are compiled in Table 4. The change of main transition temperature of DMPC–drug (in an ionic environment) and DPPE–drug mixtures did not depend linearly on the physicochemical parameters of the drugs. In other cases significant linear correlations were found, the selected independent variables explain about 35–85% of the change of phase transition parameters (see $r^2$ values). The changes of the main transition temperatures of DMPC–drug and DPPE–drug mixtures depended mainly on the lipophilicity of the drugs (see $b'$ % values of equations 1 and 4). This result supports our previous conclusion that the hydrophobic moiety of drugs insert into the fatty acid chains disordering membrane structure. However, the hydrophilic surface area of drugs also influences significantly the interaction. In the case of the basic DPPE the effect is related to the hydrophilic surface area determined on basic surface whereas with the zwitterionic DMPC the effect is correlated with the hydrophilic surface area determined on acidic surface. We assume that the basic propargylamine group interacts with the head groups of phospholipids and the strength of interaction depends on the polarity of the corresponding hydrophilic substructures. The impact of physicochemical parameters on the peak widths of various phospholipids lends support to the hypothesis that both hydrophobic and hydrophilic forces are involved in the phospholipid–drug interactions.

**Table 4**
Correlation between the phase transition characteristics of phospholipid–drug mixtures and the physicochemical parameters of drugs. Results of stepwise regression analysis

(1) $T_m$ (DMPC) = $a + b_1x_1 + b_2x_2$
(2) $\Delta T_{1/2}$ (DMPC) = $a + b_1x_3$
(3) $\Delta T_{1/2}$ (DMPC + NaCl) × $a + b_1x_4 + b_2x_5 + b_3x_6$
(4) $T_m$ (DPPE) = $a + b_1x_2 + b_2x_7$
(5) $\Delta T_{1/2}$ (DPPE) = $a + b_1x_5 + b_2x_6 + b_3x_8$
(6) $\Delta T_{1/2}$ (DPPA) = $a + b_1x_9$

| Parameter | Equation no. | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| $a$ | 0.72 | 1.97 | −1.05 | 1.53 | −2.92 | 0.41 |
| $b_1$ | 1.45 | −2.03 | −0.98 | −1.81 | 0.44 | $-9.12 \times 10^{-4}$ |
| $s_{b1}$ | 0.48 | 0.39 | 0.30 | 0.37 | 0.14 | $3.19 \times 10^{-4}$ |
| $b_2$ | −1.41 | — | 0.31 | $3.50 \times 10^{-2}$ | 0.70 | — |
| $s_{b2}$ | 0.32 | — | 0.12 | $1.16 \times 10^{-2}$ | 0.16 | — |
| $b_3$ | — | — | 0.25 | — | 0.80 | — |
| $s_{b3}$ | — | — | 0.12 | — | 0.14 | — |
| $b_1'$ % | 40.98 | — | 46.08 | 61.88 | 23.47 | — |
| $b_2'$ % | 59.02 | — | 31.06 | 38.12 | 34.40 | — |
| $b_3'$ % | — | — | 22.86 | — | 42.13 | — |
| $r^2$ | 0.6026 | 0.6504 | 0.8473 | 0.6320 | 0.8099 | 0.3533 |
| $F$ | 10.61 | | 24.05 | 12.02 | 18.46 | — |

$x_1$ = specific hydrophilic surface area on acidic surface (solvent shows no hydrogen bonding capacity).
$x_2$ = lipophilicity under acidic conditions.
$x_3$ = adsorption capacity on acidic surface (solvent shows no hydrogen bonding capacity).
$x_4$ = adsorption capacity on acidic surface (solvent shows hydrogen bonding capacity).
$x_5$ = specific hydrophobic surface area on acidic surface.
$x_6$ = adsorption capacity on basic surface (solvent shows no hydrogen bonding capacity).
$x_7$ = specific hydrophilic surface area on basic surface (solvent shows no hydrogen bonding capacity).
$x_8$ = lipophilicity under basic conditions.
$x_9$ = specific hydrophilic surface area on basic surface (solvent shows hydrogen bonding capacity).

**Table 5**
Similarities and dissimilarities between the phase transition parameters of phospholipids. Results of principal component analysis

| Principal component no. | Eigenvalue | Variance explained (%) |
|---|---|---|
| 1 | 3.93 | 49.13 |
| 2 | 2.07 | 25.81 |
| 3 | 1.05 | 13.07 |
| 4 | 0.43 | 5.37 |

| | Principal component loadings | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| $T_m$ (DMPC) | −0.84 | −0.04 | 0.40 | 0.04 |
| $\Delta T_{½}$ (DMPC) | 0.49 | 0.80 | 0.21 | 0.15 |
| $T_m$ (DMPC + NaCl) | 0.38 | −0.03 | 0.89 | −0.15 |
| $\Delta T_{½}$ (DMPC + NaCl) | 0.51 | 0.79 | −0.04 | 0.26 |
| $T_m$ (DPPE) | −0.96 | 0.03 | 0.03 | 0.19 |
| $\Delta T_{½}$ (DPPE) | 0.91 | 0.01 | −0.17 | −0.26 |
| $T_m$ (DPPA) | −0.72 | 0.61 | −0.05 | −0.06 |
| $\Delta T_{½}$ (DPPA) | 0.58 | −0.64 | 0.09 | 0.45 |

The results of principal component analysis are compiled in Table 5. Three principal components explain the overwhelming majority (88%) of variance. This result indicates that the information content of the eight phase transition parameters can be expressed by three background variables. Unfortunately, PCA does not define these background variables as concrete physical entities, only states its mathematical possibility. To ascribe concrete physicochemical meaning to the background variables, linear correlations have to be calculated between the background variables and the physicochemical parameters of the concrete variables [17, 18]. On the basis of the distribution of the PC loadings of the phase transition parameters among the principal components no clear-cut clustering can be observed. This finding indicates that the interaction of MAO inhibitory drugs is not selective enough and does not depend on the type of phospholipid. We have to emphasize that this conclusion is valid only for the phospholipid classes in the experiment and its extrapolation may lead to serious errors.

The phase transition parameters are widely distributed on the two-dimensional nonlinear map of PC loadings (Fig. 1) which means that each phospholipid interacts differently with the MAO inhibitory drugs. The main transition temperatures and peak half widths form two loose clusters indicating the different information content of both parameters. This finding is somewhat surprising because both parameters are the indicators of the ordering of fatty acid chains of phospholipids. However,

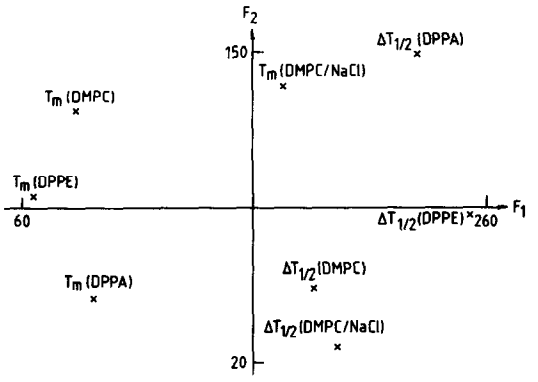



**Figure 1**
Two-dimensional nonlinear map of principal component loadings. Number of iterations: 118; maximum error: $2.15 \times 10^{-2}$.

their exact physicochemical meaning has not been elucidated in detail, and the data suggest that they measure the different aspects of packing of fatty acid chains.

Only compounds 6 and 12 are separated from the others on the two-dimensional nonlinear map of PC variables (Fig. 2). These two drugs have the highest impact on the phase transition parameters of each phospholipids. As only these compounds contain chlorine atoms, we assume that the presence of chloro substituent accounts for the membrane damaging effect of MAO inhibitory drugs.

It can be concluded from our data that the bioactive propargylamine derivatives interact with membrane phospholipids and the interaction depends both on the type of phospholipid and the hydrophobic and hydrophilic character of the derivatives. The drug–phos-

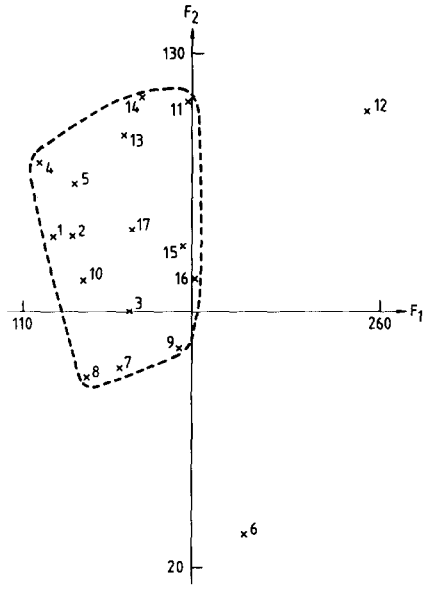



**Figure 2**
Two-dimensional nonlinear map of principal component variables. Number of iterations: 124; maximum error: $2.45 \times 10^{-2}$. Numbers refer to monoamine oxidase inhibitory drugs in Table 1.

pholipid interaction may account for the biological activity of drugs either by disturbing various membrane functions or by modifying the phospholipid arrangement around the monoamine oxidase molecules resulting in decreased enzyme activity.

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