# Synthesis of a phosphocholine based lipid for improved micellar LC based in vitro predictions of human intestinal absorption and blood-brain barrier partitioning

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## INTRODUCTION

New pharmaceutical compound
- High oral bioavailability is desired
  - Good oral absorption is the first requirement
    - Interest in predicting human intestinal absorption (HIA) [1]
- Effect on the central nervous system (CNS)?
  - Several mechanisms regulating drug permeability to the brain
    - Blood-brain barrier (BBB) is the most important
      - High BBB permeability ⟷ indication for CNS effect
      - Common measure: $\log BB = \log(\frac{C_{brain}}{C_{blood}})$ [2]

In vitro HIA and log BB prediction using Micellar liquid chromatography (MLC)
- RPLC; surfactant above critical micellar concentration (CMC) in mobile phase
- Secondary equilibrium (Figure 1) [3]
  - Stationary phase ⟷ bulk solvent
  - Micelles ⟷ bulk solvent
- Retention time + descriptors ⟶ model

Thus far in MLC: SDS, Brij35, CTAB were used as surfactants.
- Not comparable to membrane lipids

⟹ Miltefosine (Figure 2) is presented here as an alternative MLC-surfactant

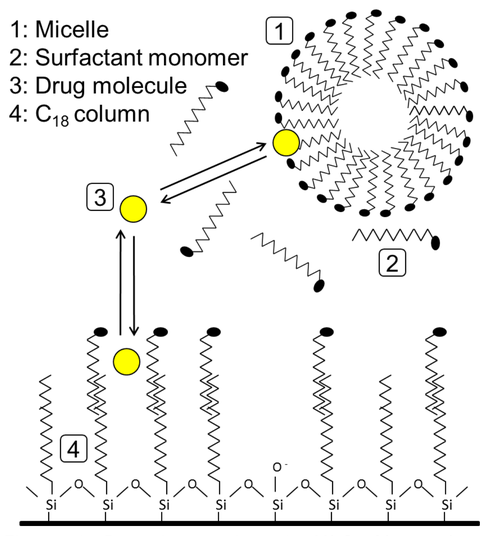


**Figure 1: Drug interactions in MLC. Above the CMC, retention depends on interactions with the modified stationary phase and with the micelles present in the mobile phase.**

Model construction based on log k + computed descriptors
- HIA prediction ⟷ experimental HIA values
- Log BB prediction ⟷ experimental log BB values
- Finally: model evaluation

## EXPERIMENTAL

### *Synthesis of miltefosine*

The synthesis route (Figure 2) was slightly modified from a previously reported procedure by Zhang et al. [4]. HPLC-TOF-MS, $^{1}$H-NMR and $^{13}$C-NMR were used for structure confirmation.

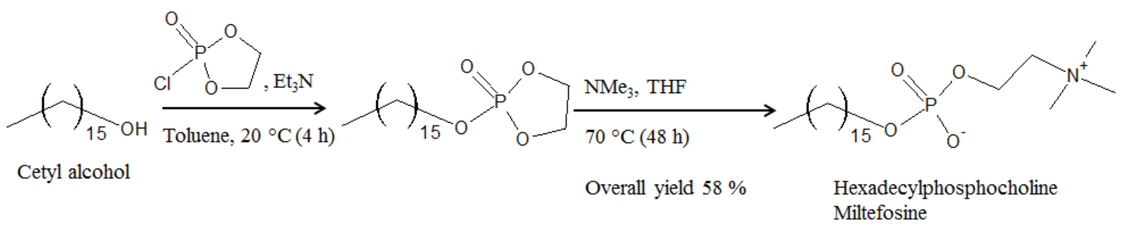


**Figure 2: Synthesis of miltefosine.**

### *MLC*

0.01 M miltefosine was dissolved in the mobile phase. The pH was adjusted with a phosphate buffer at pH 7.4. The osmotic pressure was reproduced by addition of NaCl (9.20 g/L). Column & flow rate: GraceSmart $C_{18}$ column (3 µm, 150 mm x 2.1 mm) at 37 ° C, flow rate 0.2 ml/min.

### *HIA and log BB*

The retention factors (k) of the compounds were determined
Several molecular descriptors were added to the model
- Total molar charge
- Molar volume
- Log P
- pH solubility profile
- Human intestinal absorption
- Hydrogen bond donor
- Molecular weight
- Parachor
- Log D 7.4
- Plasma protein binding
- Polar surface area
- Molecular surface area
- Molar refractivity
- Polarizability
- Intrinsic aqueous solubility
- Ames test mutagenic index
- Hydrogen bond acceptor

Partial least squares (PLS) regression
- Correlation coefficient (R) between actual (in vivo) and predicted values
- Selecting the most relevant descriptors: monitor effect on the leave-one-out cross-validation (LOOCV) regression coefficients upon systematic removal and/or reinsertion of all descriptors from the models
  - Both models: remove
    - Molar refractivity
    - Log P
    - Ames test mutagenic index
    - Molar volume
    - Log D 7.4
    - Hydrogen bond donor

## RESULTS & DISCUSSION

To illustrate the retention behavior in purely aqueous MLC with 0.01 M miltefosine, some chromatograms are presented in Figure 3.

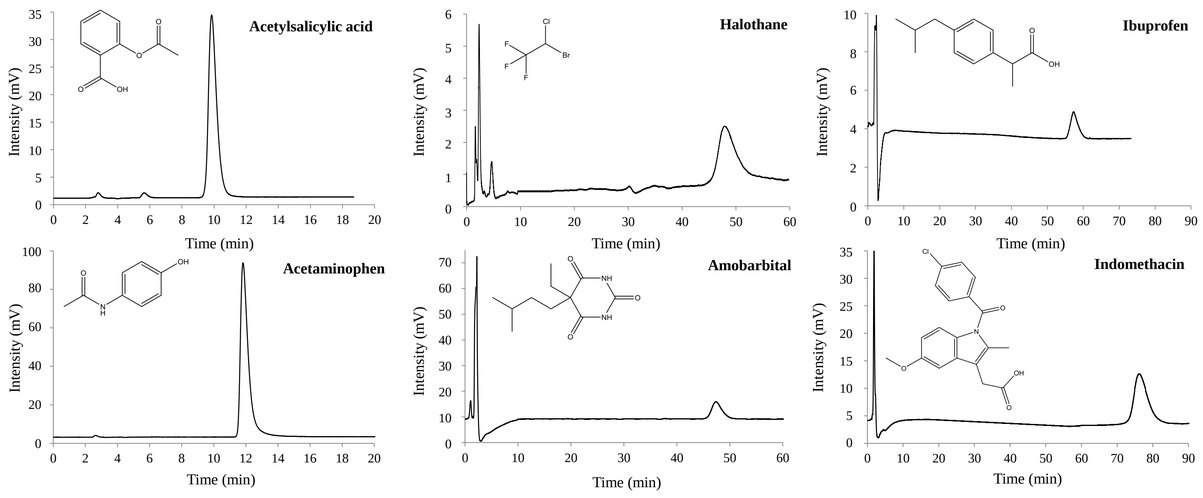


**Figure 3: Chromatograms for some compounds using MLC with miltefosine as surfactant**

The results from the PLS and LOOCV regressions before and after elimination of superfluous molecular descriptors are presented in Table 1. The large difference in correlation coefficient before optimization is an indication of overfitting in the model. By removing unnecessary descriptors, the overfitting was reduced a lot. The final correlation coefficient of HIA (0.7175; based on 36 compounds) was lower than that for log BB (0.7849; based on 48 compounds). For both predictions, data provided by MLC with miltefosine proved to contribute in a positive way.
The correlation between actual and predicted HIA and log BB values is illustrated in Figure 4 before and after optimization. Although there are a few outsiders, the predicted values for most compounds are close to the actual (in vivo) determined values.

**Table 1: Correlation coefficients between actual and predicted HIA and log BB values using PLS and LOOCV before and after optimization of molecular descriptors.**

**Before optimization**

| | HIA | Log BB |
|---|---|---|
| R(PLS) | 0.8237 | 0.8827 |
| R(LOOCV) | 0.3666 | 0.5298 |

**After optimization**

| | HIA | Log BB |
|---|---|---|
| R(PLS) | 0.7991 | 0.8484 |
| R(LOOCV) | 0.7175 | 0.7849 |

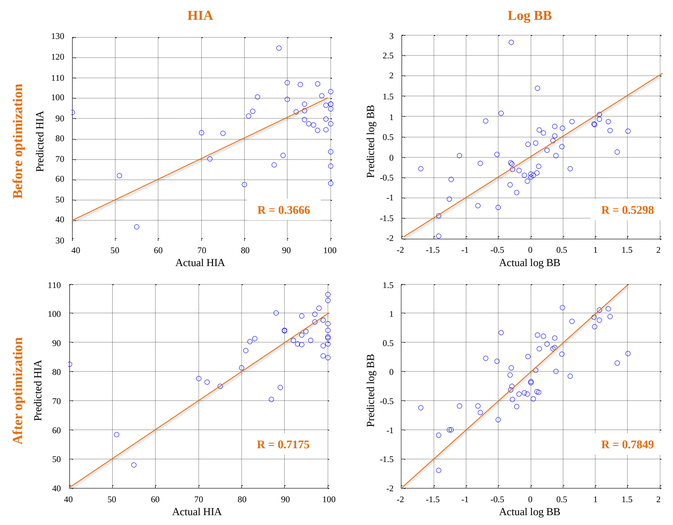


**Figure 4: Visual representation of the correlation between actual and predicted log BB values using the LOOCV method before and after elimination of superfluous molecular descriptors.**

### *Prediction of HIA and log BB values*

Each PLS regression leads to an equation, generally written as $Y = b_0 + b_1 X_1 + b_2 X_2 + \ldots + b_n X_n$, where Y can be HIA or log BB, and $X_1 \ldots X_n$ are the molecular descriptors. The coefficients ($b_0 \ldots b_n$) for the two models are divers, reflecting the difference between HIA and BBB permeation.

## CONCLUSION

- **The log BB model performed better compared to HIA prediction, although data provided by MLC with miltefosine as surfactant contributed in a positive way to both models.**
- **This approach shows potential as an alternative or complementary MLC strategy to predict in vivo behavior.**
- **Additional research, using a variety of (phospho)lipids as surfactant for MLC, might be very interesting, since this could better mimic the composition of biological membranes.**

## REFERENCES

[1] Hou TJ, Wang JM, Zhang W, Xu XJ (2007) J Chem Inf Model 47 (1):208-218.
[2] Fu XC, Wang GP, Shan HL, Liang WQ, Gao JQ (2008) Eur J Pharm Biopharm 70 (2):462-466.
[3] Molero-Monfort M, Escuder-Gilabert L, Villanueva-Camañas RM, Sagrado S, Medina-Hernández MJ (2001) J Chrom B 753 :225-236
[4] Zhang QH, Horst R, Geralt M, Ma XQ, Hong WX, Finn MG, Stevens RC, Wuthrich K (2008) J Am Chem Soc 130 (23):7357-7363.