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 (Brain / Blood)

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

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, 1H-NMR and 13C-NMR were used for structure confirmation.

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 C18 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
- 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 reactivity
- Log P
- Ames test mutagenic index
- 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.

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 outliers, the predicted values for most compounds are close to the actual (in vivo) determined values.

 Prediction of HIA and log BB values
Each PLS regression leads to an equation, generally written as Y = b0 + b1 x1 + b2 x2 + ... + b8 x8. Where Y can be HIA or log BB, and x1 ... x8 are the molecular descriptors. The coefficients (b0, ..., b8) for the two models are diverse, reflecting the difference between HIA and BB 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