ECOTOXICITY OF DIHYDROPYRIDINES, CALCIUM CHANNELS BLOCKERS: *IN SILICO* SURVEY

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Abstract

There is growing evidence that the presence of pharmaceuticals in the environment results in exposure of biota, posing a potential threat to ecosystem health. Assessing the bioaccumulation of active pharmaceutical ingredients is critical, as it provides insight into the extent to which emerging contaminants may persist and concentrate within organisms. In this study, the ecotoxicological endpoints of selected twenty dihydropyridines were determined using the online ADMETlab 3.0 online programme. In addition, their polarity, solubility, and lipophilicity were determined based on their structure, followed by examination of the possible association of their physico-chemical properties and ecotoxicological endpoints. The obtained results suggested that their bioaccumulation factor is strongly positively associated with the lipophilicity of the observed dihydropyridines and negatively with their solubility and polarity, respectively. Moreover, the 48h 50% inhibition growth concentration of Tetrahymena pyriformis, the 48h-LC_{50} of Daphnia magna and 96h-LC_{50} of Fathead minnow for the analysed compounds were governed positively by the lipophilicity and negatively by their aquatic solubility, respectively, whilst the influence of the polarity of the molecules was not statistically significant. Further studies require experimental confirmation of the ecotoxicological potential of dihydropyridines as one of the most prescribed pharmaceuticals in the treatment of cardiovascular diseases.

Introduction

Most of pharmaceuticals excreted in urine are not effectively removed by wastewater treatment plants and consequently enter aquatic environments. Several active pharmaceutical ingredients (APIs) have been shown to exert toxic effects on aquatic organisms. The continuous prescription of APIs for various chronic disease contribute to the constant release of active pharmaceutical ingredients into waterways through wastewater treatment plants effluent. This sustained discharge raises serious environmental concerns, as APIs have been linked to a wide range of harmful impacts on both aquatic flora and fauna [1]. Thus, there is an urgent need for robust environmental risk assessment (ERA) of APIs. This has prompted the development of new approach methodologies (NAMs), which aim to generate hazard and risk information without the use of animals, in line with ethical frameworks of animal protection. Among these NAMs, computational or *in silico* methods, especially quantitative structure–activity relationship (QSAR) models, have become crucial, as they can predict toxicity across untested chemical-species combinations [2]. A QSAR model seeks to quantify how variations in chemical structure influence a molecule's biological activity or physico-chemical properties. These models are highly versatile, and can be applied to a broad spectrum of endpoints including pharmacological effects, toxicological outcomes, biokinetic behaviours, and physical or chemical characteristics. Finally, QSARs support chemical safety assessment by predicting

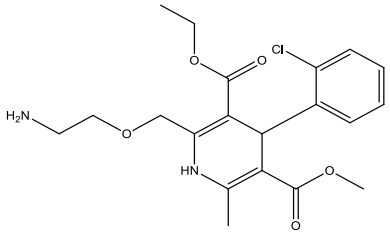
toxicity and biokinetics for both human health and environmental safety, enabling individual compound risk assessment and thus potentially reducing reliance on animal experiments [3].
Dihydropyridines as type of calcium channel blockers (CCBs) are among the most often prescribed drugs for the treatment of hypertension [4]. CCBs inhibit the influx of calcium by binding to L-type, long-acting voltage-gated calcium channels found in the heart, vascular smooth muscle, and pancreas. Dihydropyridines have minimal direct impact on the myocardium and act mainly as peripheral vasodilators at therapeutic doses. This makes them effective in treating conditions like hypertension, vasospasm following intracranial haemorrhage, and migraines [5], but also attributes to their harmful influence on various species in the environment. In this paper, the ecotoxicological potential of twenty dihydropyridines (Figure 1) has been examined *in silico*, with evaluation of the change in their physico-chemical properties on the obtained ecotoxicological endpoints.

Experimental

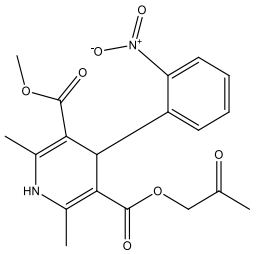
In this paper in ADMETlab 3.0 online program (https://admetlab3.scbdd.com/) was used for evaluating the ecotoxicological potential of 20 dihydropyridines (Figure 1). Two-dimensional structures of the examined compounds were obtained by ChemDraw Professional as Simplifies Molecular-Input Line-Entry System, SMILES and used for predicting their properties. Their polarity expressed as total polar surface area (TPSA), solubility given as logS as well as lipophilicity obtained in form of partition coefficients logP and logD were determined. The ecotoxicological potential of each compound was evaluated as bioconcentration factors (BCF), IGC_{50}, $LC_{50}FM$ and $LC_{50}DM$, respectively. For all above predictors the unit is negative value of log10[(mg/L)/(1000*MW)]. BCF are used for considering secondary poisoning potential and assessing risks to human health via the food chain. IGC_{50} presents the 50% inhibition growth concentration after 48h of Tetrahymena pyriformis. The concentration that kills 50% of Fathead minnow after 96h is given as $LC_{50}FM$, while the concentration that kills 50% of Daphnia magna after 48h is presented as $LC_{50}DM$.

Results and discussion

Bioaccumulation serves as a key parameter in environmental risk assessment, particularly for evaluating long-term ecological impacts. This focus is essential not only for understanding population-level effects but also for assessing risks to individual organisms across trophic levels [6]. The BCF for the compounds analysed varied between 0.763 and 2.134 with mean value of 1.426 ± 0.299 (Figure 2). Although, there are no data for BCF among humans, there is a list of BCF values expressed in wet weight or dry weight bases in L/kg for different aquatic species. The variation of bioaccumulation for different pharmaceuticals suggested BCF values such as 3981 L/kg in fish for cefotaxime, 49,000 L/kg for ibuprofen in trout, with up to 209,500 fluoxetine in shrimps [7].
Tetrahymena pyriformis a protozoan model organism used for environmental toxicology analyses. Daphnia magna (DM), a small freshwater crustacean and Pimephales promelas (fathead minnows, FM), a small fish model, are routinely used for regulatory programs aimed at assessing potential risks of chemicals including pharmaceuticals. The 48h 50% inhibition growth concentration of Tetrahymena pyriformis, IGC_{50} was in the interval of 3.571 and 4.714, while $LC_{50}DM$ varied between 4.315 and 5.863 for the analysed dihydropyridines (Figure 2). The experimental data for verapamil, a non-dihydropyridines, calcium channel blocker indicated that 48h-LC_{50} was 7.04 mg/L [8]. Finally, the $LC_{50}FM$ levels for the selected dihydropyridines were between 4.896 and 6.332 (mean value of 5.673 ± 0.423, Figure 2). Experimental data for verapamil suggested that although FM survival was not impacted by verapamil exposure, the growth was significantly decreased at 600 μg/L [9].



(1) Amlodipine



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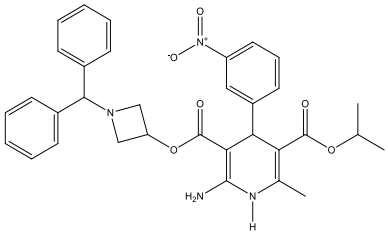

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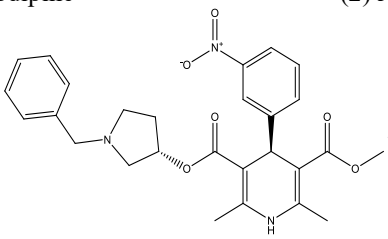
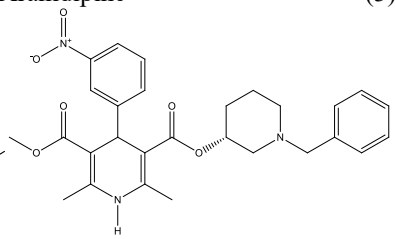
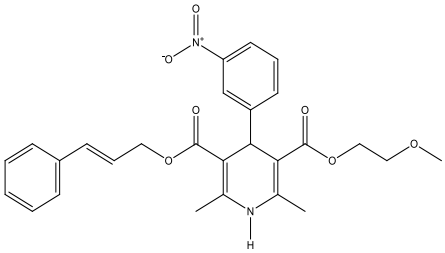
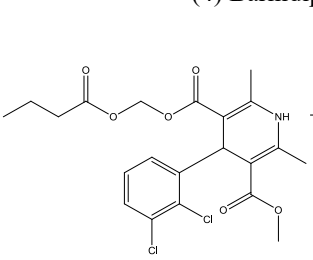
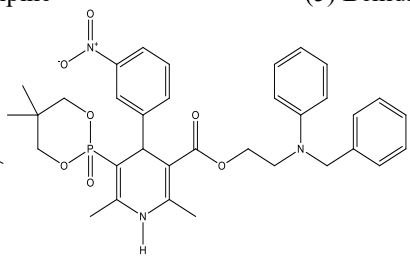


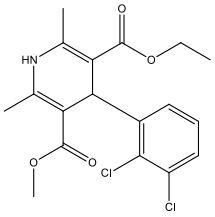


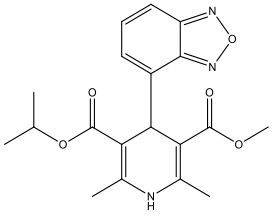


(4) Barnidipine (5) Benidipine (6) Cilnidipine

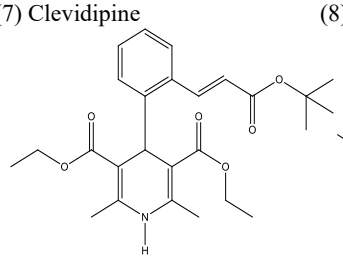


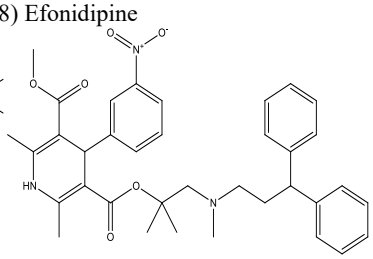


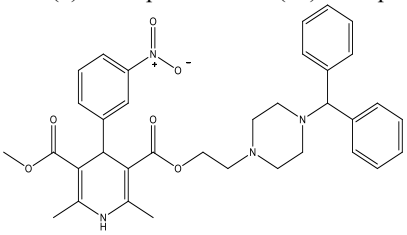




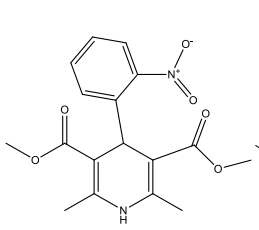
(7) Clevidipine (8) Efonidipine (9) Felodipine (10) Isradipine

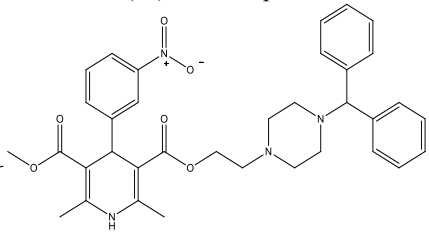


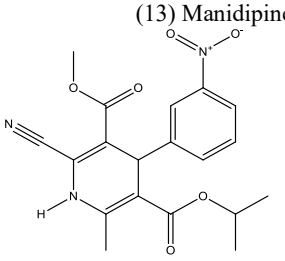




(11) Lacidipine (12) Lercanidipine (13) Manidipine







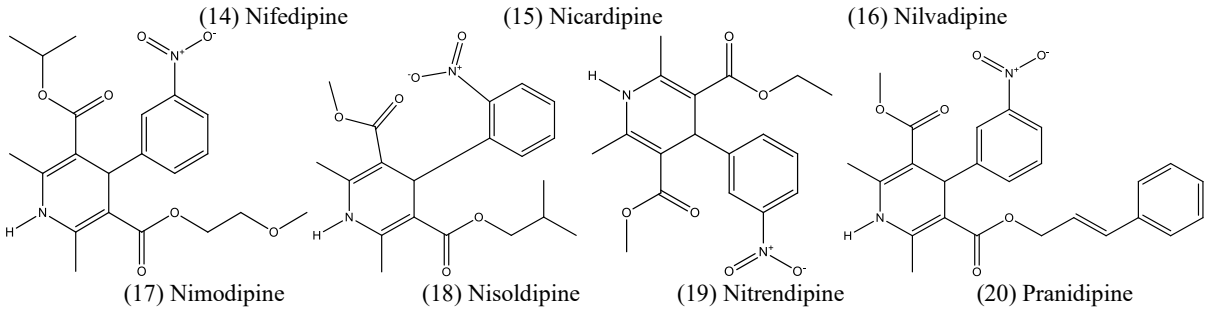


Figure 1. Structures of the analysed dihydropyridines

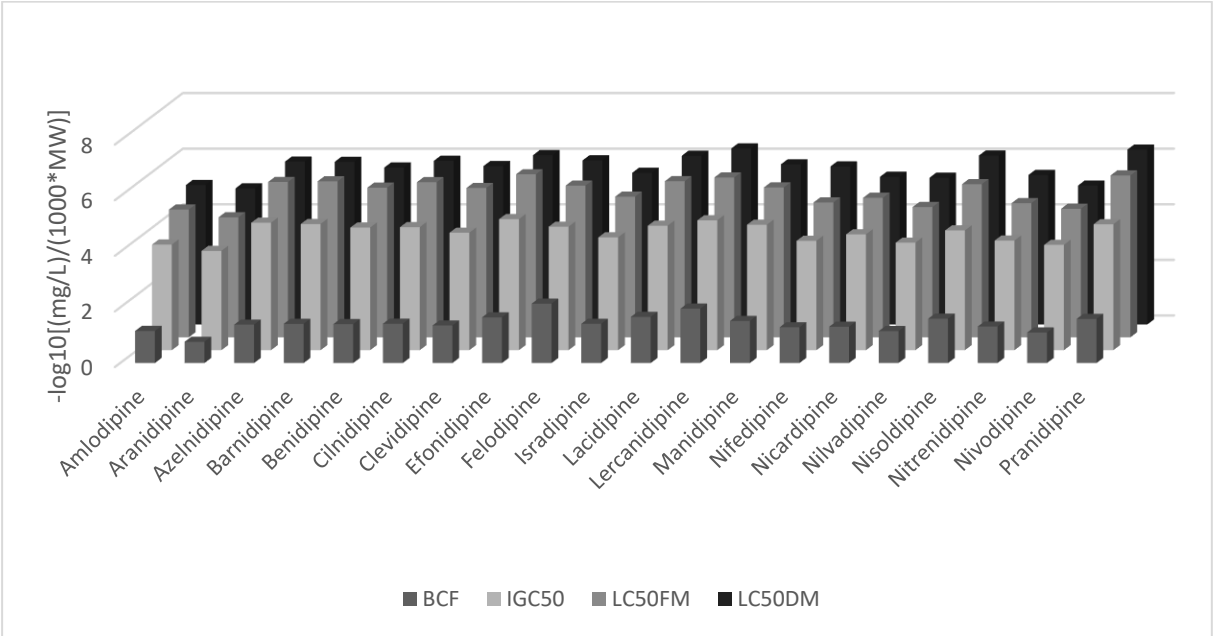
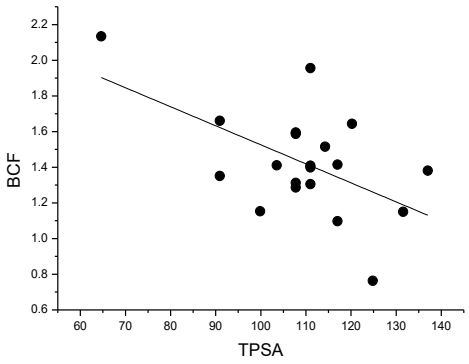
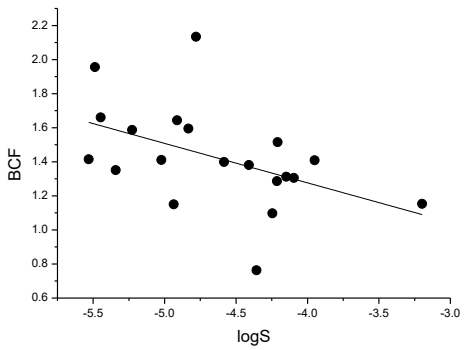


Figure 2. *In silico* evaluated ecotoxicological endpoints for the selected dihydropyridines

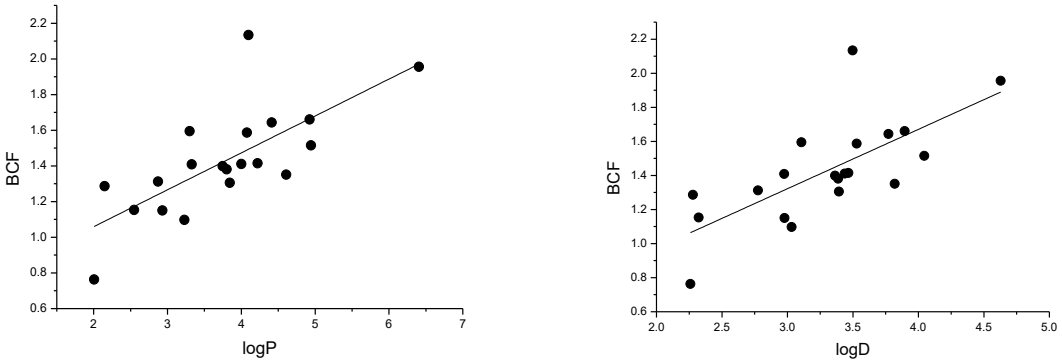
The BCF values seem to be governed negatively by the polarity, TPSA (r^2=0.268, p=0.011, Figure 3a), and the solubility, logS (r^2=0.182, p=0.034, Figure 3b), respectively. The association of BCF with lipophilicity of the compounds (r^2=0.494, p=3.30×10^{-4} with logP, r^2=0.472, p=4.95×10^{-4}, with log D) was directly proportional (Figures 3c-d).



a)



b)



c) d)

Figure 3. The association of BCF with a) TPSA, b) logS, c) logP and d) logD of the analysed dihydropyridines

The IGC_{50}, $LC_{50}FM$ and $LC_{50}DM$ were associated only with solubility and lipophilicity of the analysed compounds, while the influence of the polarity on these values was not statistically significant. Namely, IGC_{50} was negatively correlated with solubility, log S (r^2=0.163, p=0,044) and positive with lipophilicity (r^2=0.562, p=8.484×10^{-5} with log P; r^2=0.580, p=5.782×10^{-5} with log D, respectively). Solubility, logS was inversely associated with both $LC_{50}FM$ and $LC_{50}DM$ (r^2=0.275, p=0.010, r^2=0.349, p=0.004), respectively. The positive proportionality between $LC_{50}FM$ and logP (r^2=0.519, p=2.054×10^{-4}) and logD (r^2=0.535, p=1.483×10^{-4}), respectively as well as $LC_{50}DM$ positive relationship with logP (r^2=0,437, p=9.075×10^{-4}) and logD (r^2=0.435, p=9.48×10^{-4}), respectively was in accordance with the literature data. Namely, lipophilicity was recognized as a suitable predictor of bioconcentration of various pharmaceuticals in fish [10]. However, other studies indicated lack of association between lipophilicity of pharmaceuticals, within a specific category or across all classes, and their acute toxicity to individual species or groups of organisms. Despite these findings, the best relation between measured and estimated lipophilicity of pharmaceuticals and acute toxicity was reported towards Daphnia magna [11]. The inverse influence of solubility on the toxic potential of the observed molecules on contrary to their solubility is in line with the negative connection between a molecule's aquatic solubility and lipophilicity: higher lipophilicity generally indicates lower aquatic solubility, and vice versa [12].

Conclusion

The ecotoxicological endpoints can be determined *in silico* due to advanced new methodology techniques that apply machine learning. Lipophilicity is the crucial factor that governed the ecotoxicological endpoints of twenty selected dihydropyridines, while the aquatic solubility and polarity have exerted less emphasized influence. There is rapid concern regarding the influence of pharmaceuticals including dihydropyridines on aquatic environment.

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