UNIWERSYTET MARII CURIE-SKŁODOWSKIEJ W LUBLINIE

Szkoła Doktorska Nauk Ścisłych i Przyrodniczych

Dziedzina: nauki ścisłe i przyrodnicze
Dyscyplina: nauki chemiczne

Anna Śliwińska
nr albumu: 299687

Zastosowanie chromatografii cieczowej i metodyki Quantitative Structure (Retention)–Activity Relationships do przewidywania właściwości farmakokinetycznych nowych substancji organicznych rozważanych jako potencjalne leki i środki ochrony roślin

Application of liquid chromatography and Quantitative Structure (Retention)–Activity Relationships methodology to predict the pharmacokinetic properties of new organic substances considered as potential drugs and plant protection products

LUBLIN, 2023

Składam serdecznie podziękowania mojej promotor,
dr hab. Małgorzacie Janickiej, prof. UMCS
za nieocenioną pomoc podczas prowadzenia badań,
życzliwość, cenne uwagi i poświęcony mi czas.

Dziękuję pracownikom Katedry Chemii Fizycznej, szczególnie
prof. dr hab. Irenie Malinowskiej *oraz* **dr. Markowi Studzińskiemu**
za stworzenie przyjaznej, zachęcającej do pracy atmosfery w laboratorium.

Rodzinie i Przyjaciołom,
za wyrozumiałość i cierpliwość.

Szczególne podziękowania składam mojemu **Mężowi**,
za jego nieustanne wsparcie i wiarę we mnie.

Spis treści

<u>Wykaz publikacji wchodzących w skład cyklu doktorskiego</u>

D1. Janicka, M., **Mycka, A.**, Sztanke, M., & Sztanke, K. (2021). Predicting pharmacokinetic properties of potential anticancer agents via their chromatographic behavior on different reversed phase materials. *International Journal of Molecular Sciences*, *22*(8), 4257-4270. https://doi.org/10.3390/ijms22084257

IF = 6,208; PK_{MEiN} = 140

D2. Janicka, M., & **Śliwińska, A.** (2022). Quantitative retention (structure)-activity relationships in predicting the pharmaceutical and toxic properties of potential pesticides. *Molecules*, *27*(11), 3599-3618. https://doi.org/10.3390/molecules27113599

IF = 4,927; PK_{MEiN} = 140

D3. Janicka, M., **Śliwińska, A.**, Sztanke, M., & Sztanke, K. (2022a). Predicting pharmacokinetic properties of potential anti-cancer agents using micellar thin-layer chromatography. *JPC – Journal of Planar Chromatography – Modern TLC*, *35*, 265-272. https://doi.org/10.1007/s00764-022-00174-4

IF = 1,088; PK_{MEiN} = 20

D4. Janicka, M., **Śliwińska, A.**, Sztanke, M., & Sztanke, K. (2022b). Combined micellar liquid chromatography technique and QSARs modeling in predicting the blood-brain barrier permeation of heterocyclic drug-like compounds. *International Journal of Molecular Sciences*, *23*(24), 15887-15914. https://doi.org/10.3390/ijms232415887

IF = 6,208; PK_{MEiN} = 140

Sumaryczny IF (2023): 18,431
Sumaryczna liczba punktów MEiN (2023): 440

Wykaz stosowanych skrótów

α – polaryzowalność

AD – obszar zastosowania modelu

BMC – Biopartitioning Micellar Chromatography

Brij 35 – eter laurylowy glikolu polioksyetylenowego (23)

cmc – krytyczne stężenie micelizacji

CTAB – bromek cetylotrimetyloamoniowy

Caco-2 – parametr wchłaniania w jelitach

$f_{u,brain}$ – wolna (niezwiązana) frakcja w mózgu

$f_{u,plasma}$ – wolna (niezwiązana) frakcja w osoczu

HBA – liczba akceptorów wiązania wodorowego

HBD – liczba donorów wiązania wodorowego

IAM – immobilizowana sztuczna membrana

LADME - uwolnienie, wchłanianie, dystrybucja, metabolizm oraz wydalanie/eliminacja substancji bioaktywnej

LD – dawka śmiertelna

log BB – logarytm ze stałej podziału w układzie krew-mózg

log k – logarytm ze współczynnika retencji w technikach kolumnowych

log K – logarytm ze stałej podziału

log K_{HSA} – logarytm ze stałej wiązania z albuminą

log K_p – logarytm ze stałej przenikania przez skórę z roztworów wodnych

log K_{sc} – logarytm ze stałej podziału w układzie woda-skóra

log $K_{w/cell}$ – logarytm ze stałej przenikania do komórek roślinnych

log P_{HSA} – logarytm ze współczynnika podziału w układzie woda-albumina

log $P_{o/w}$ – logarytm ze współczynnika podziału w układzie n-oktanol/woda

log $P_{w/pc}$ – logarytm ze współczynnika podziału w układzie woda-kutykula

MLC – micelarna chromatografia cieczowa

MLR – wielokrotna regresja liniowa

MSE – średni błąd kwadratowy

MW – masa cząsteczki

NRB – liczba wiązań rotacyjnych

OECD – Organizacja Współpracy Gospodarczej i Rozwoju

p – prawdopodobieństwo testowe

P – parachora

PCA – analiza głównych składowych

PRESS – przewidywany błąd resztowy sumy kwadratów

QRARs – ilościowe zależności między retencją a aktywnością biologiczną

QSARs – ilościowe zależności między strukturą a aktywnością biologiczną

R – współczynnik korelacji

R^2 – współczynnik determinacji

R_M – parametr retencji w technikach planarnych

RP LC – chromatografia cieczowa w odwróconym układzie faz

sd – odchylenie standardowe

SDS – dodecylosiarczan sodu

TLC – chromatografia cienkowarstwowa

TPSA – topologiczne pole powierzchni polarnej

V_d – objętość dystrybucji

VIF – współczynnik wariancji inflacji

v/v – ułamek objętościowy

Uzasadnienie wyboru tematu

Współczesna nauka, poza realizacją badań podstawowych, powinna odpowiadać na potrzeby społeczeństwa. Niestety, najbardziej istotne problemy pozostają niezmienne od wielu lat i wciąż istnieje potrzeba ich rozwiązania. Za główne globalne problemy społeczne uznaje się: ubóstwo, zmiany klimatyczne, głód i braki żywności, kryzys migracyjny, wpływ pandemii na gospodarkę, choroby i brak dostępu do opieki zdrowotnej (*Top 20 Current Global Issues We Must Address*, n.d.). Przeprowadzane badania naukowe powinny przyczyniać się do podniesienia poziomu życia oraz rozwoju społeczeństwa. Poszukiwanie, synteza i analiza substancji aktywnych biologicznie może stanowić odpowiedź na część z przedstawionych problemów. Polepszenie jakości ludzkiego życia jest możliwe dzięki takim substancjom jak nowe leki, pozwalające ograniczyć ból, pokonać nowotwory, czy choćby przyspieszać zwalczanie różnorodnych infekcji. Ograniczanie problemu głodu i dostępności jedzenia jest ściśle powiązane z wykorzystywaniem pestycydów, zwłaszcza w krajach, gdzie plony są ubogie. Oczywiście, stosowane związki ochrony roślin powinny jak w najmniejszym stopniu zanieczyszczać środowisko i nie wpływać na jego bioróżnorodność.

Substancje biologicznie aktywne oddziałują na każdy żywy organizm. Synteza tego typu związków powinna prowadzić do uzyskania substancji, których właściwości pożądane znacznie przewyższają niepożądane. Reguła Lipińskiego, zmodyfikowana, rozszerzona i bardziej szczegółowa, ale wciąż powszechnie stosowana, pozwala na zaplanowanie kierunku syntezy, który przyniesie oczekiwane efekty, tj. pożądaną aktywność biologiczną substancji. Według oryginalnie zaprezentowanej reguły związek chemiczny powinien spełniać następujące kryteria: w jego cząsteczce musi być nie więcej niż pięć donorów i nie więcej niż dziesięć akceptorów wiązania wodorowego, masa cząsteczki nie może przekraczać 500 Da, a wyliczona wartość parametru log $P_{o/w}$ nie może być wyższa niż pięć (Lipinski et al., 2001). Wymagania stawiane potencjalnie bioaktywnym substancjom zostały uszczegółowione i rozszerzone w celu zwiększenia prawdopodobieństwa aktywności i dostępności biologicznej związków. Jedne z pierwszych uściśleń ustalały przedział wartości współczynnika podziału log $P_{o/w}$ od -0,4 do 5,6; zakres masy molowej od 180 do 480 Da oraz dodatkowo wprowadzały ograniczenie liczby atomów w badanej cząsteczce od 20 do 70, a także zakres refrakcji molowej od 40 do 130 m^3 mol^{-1} (Ghose et al., 1999). Według Vebera i wsp. o dobrej biodostępności po podaniu doustnym decydują głównie dwa kryteria: obecność dziesięciu lub mniej wiązań rotacyjnych w cząsteczce oraz pole powierzchni polarnej nie większe od 140 Å^2

(Veber et al., 2002). Stosowanie przedstawionych reguł znacznie ułatwia proces poszukiwania nowych substancji leczniczych oraz środków ochrony roślin. Jednak każda z nowo uzyskanych substancji musi zostać poddana weryfikacji eksperymentalnej oraz ocenie, czy wymagane działanie faktycznie występuje. Oczywistym jest, że w dalszej części badań klinicznych trudno jest wyeliminować badania na zwierzętach, pomimo ich wątpliwej etyczności oraz wysokich kosztów. Należy jednak uczynić wszystko, żeby unikać ich w czasie badań przesiewowych, a przynajmniej znacznie je ograniczać.

Wymienione powyżej kryteria decydują o przebiegu procesów farmakokinetycznych, które zachodzą po wprowadzeniu do ustroju substancji biologicznie aktywnej. Do głównych procesów zalicza się uwolnienie substancji aktywnej, jej wchłanianie, dystrybucję, metabolizm oraz wydalanie z organizmu (LADME) (Lentz et al., 2013; Rein et al., 2013; Roberts, 2003; Ruiz-Garcia et al., 2008). W celu wywołania pożądanego efektu substancja aktywna przekracza w organizmie różne bariery biologiczne, co oznacza, że umiejętność przewidywania jej przenikania przez wspomniane bariery jest absolutnie konieczna. Substancje aktywne są zdolne do przenikania przez błony komórkowe zarówno poprzez transport aktywny, jak i znacznie częściej występujący transport bierny. W takim przypadku transport substancji poprzez membranę zależy od jej właściwości fizykochemicznych, takich jak lipofilowość, wielkość cząsteczki, współczynnik podziału, czy gradient stężeń (Grabowski, 2015).

Przenikanie poprzez barierę krew-mózg należy do najważniejszych dróg transportu, ponieważ decyduje, czy substancja będzie oddziaływać na ośrodkowy układ nerwowy. O ile takie działania powinno być wywoływane przez niektóre leki, o tyle w przypadku leków działających obwodowo przenikanie przez wspomnianą barierę jest zaliczane do działań niepożądanych. Środki ochrony roślin również nie powinny oddziaływać na ośrodkowy układ nerwowy. W obu przypadkach ocena zdolności substancji do przenikania poprzez barierę krew-mózg jest niezbędna i powinna zostać przedstawiona już na początkowym etapie badań (Ajay et al., 1999; Feher et al., 2000; Geldenhuys et al., 2015). Pomimo tego, że proces przekraczania tej bariery jest złożony, współcześnie wiadomo, że umiarkowanie lipofilowe substancje ulegają transportowi biernemu (Mouritsen & Jørgensen, 1998; Seddon et al., 2009; Wolak & Thorne, 2013). Parametr $\log BB$, wyznaczany jako stosunek stężenia substancji bioaktywnej w mózgu do jej stężenia we krwi w stanie równowagi, wykorzystywany jest do opisywania wpływu badanej substancji na ośrodkowy układ nerwowy (Iyer et al., 2002). Tradycyjny sposób jego wyznaczania zalicza się do metod drogich, pracochłonnych oraz

wymagających testowania na ludziach lub zwierzętach, dlatego współcześnie zaleca się stosowanie metod pośrednich. Podczas opisywania procesów transportu substancji oraz jej przenikania przez barierę krew-mózg wykorzystywane są również parametry $f_{u,brain}$ i $f_{u,plasma}$, charakteryzujące stężenie niezwiązanej frakcji substancji w – odpowiednio – mózgu i osoczu (Y. C. Chen et al., 2019; Fridén et al., 2010).

Zdolność substancji aktywnych biologicznie do wiązania z białkami krwi jest niezbędna do ich transportu w organizmie (Tesseromatis & Alevizou, 2008). Największy udział wśród białek występujących w osoczu ludzkim ma albumina. Informacja dotycząca wiązania analizowanej substancji z albuminą pozwala na przewidywanie jej właściwości farmakokinetycznych (Vuignier et al., 2010). Najczęściej stosowanymi parametrami do opisu tej zdolności są logarytm ze stałej wiązania albumina-substancja (oznaczany jako $\log K_{HSA}$ lub $\log K_{a,HSA}$) oraz logarytm ze współczynnika podziału substancji pomiędzy wodę a albuminę ($\log P_{HSA}$ lub $\log P_{w/HSA}$). Podczas analizowania wiązania substancji z albuminą należy pamiętać, że istnienie takiego połączenia jest zależne od możliwości przyjęcia przez substancję odpowiedniej konformacji, a więc od jej właściwości sterycznych (Fasano et al., 2005; Yamasaki et al., 2013; Yang et al., 2014).

Bardzo istotnym procesem po wprowadzeniu substancji bioaktywnej do organizmu jest wchłanianie zwrotne. Większość związków z grupy substancji leczniczych po podaniu doustnym ulega wchłanianiu dopiero w jelitach, a następnie transportowi do krwi (Azman et al., 2022; Dahlgren & Lennernäs, 2019; O'Shea et al., 2022). Powszechnie stosowanym parametrem opisującym ten proces jest *Caco-2* (Camenisch et al., 1998). Należy również pamiętać, że nie wszystkie leki są podawane doustnie. W przypadku substancji leczniczych zawartych w różnego rodzaju kremach lub maściach istotna jest znajomość parametrów charakteryzującego przenikanie przez skórę, do których należy logarytm ze stałej przenikania przez skórę z roztworów wodnych ($\log K_p$) (C. P. Chen et al., 2018; Prausnitz & Langer, 2008), czy ze stałej podziału w układzie woda-skóra ($\log K_{sc}$) (Aggarwal et al., 2015; Kerns & Di, 2003). Ważna jest także możliwość przewidywania ilości substancji aktywnej w organizmie na podstawie pomiaru jej chwilowego stężenia w osoczu, opisywana przez objętość dystrybucji (V_d) (Chan et al., 2018; Toutain & Bousquet-Mélou, 2004).

Wyznaczenie parametrów farmakokinetycznych scharakteryzowanych powyżej jest bardzo istotne zarówno dla związków o działaniu leczniczym, jak i dla środków ochrony roślin. W przypadku potencjalnych leków wymienione parametry opisują losy substancji aktywnych po ich celowym wprowadzeniu do organizmu ludzkiego (Betarbet et al., 2000;

Goulson et al., 2015; Joko et al., 2023; Matsuzaki et al., 2023). Nie można natomiast wykluczyć, że potencjalne pestycydy również dostaną się do układu pokarmowego ludzi lub zwierząt, choćby poprzez nieprzestrzeganie wymaganych okresów karencji lub niestaranne przygotowanie produktów roślinnych do spożycia. Znajomość wspomnianych parametrów dla środków ochrony roślin pozwala na ocenę ich toksyczności w stosunku do ludzi i zwierząt. Ponadto istnieje możliwość wyznaczenia parametrów farmakokinetycznych odnoszących się głównie do tej grupy bioaktywnych substancji, takich jak logarytmu ze stałej przenikania do komórek roślinnych (log $K_{w/cell}$) (Platts et al., 2000) lub ze współczynnika podziału pomiędzy wodę a nabłonek (kutykulę) rośliny (log $P_{w/pc}$) (Baur et al., 1997).

Istotnym parametrem toksyczności jest dawka śmiertelna LD_{50}, oznaczająca dawkę potrzebną do spowodowania śmierci 50% osobników danej populacji po przyjęciu substancji aktywnej wskazaną drogą. W badaniach najczęściej wykorzystuje się myszy lub szczury, a dawkę można zaaplikować doustnie, podskórnie lub dożylnie (Karlsson, 1996; Quiñones-Torrelo et al., 2001).

Aktywność biologiczna danego związku jest zależna od kilku czynników: jego lipofilowości, efektów sterycznych oraz elektronowych, czyli – najkrócej ujmując – od jego budowy. Zdolność substancji do rozpuszczania się w rozpuszczalnikach niepolarnych (lipofilowość) często okazuje się najbardziej istotną właściwością, decydującą o jej bioaktywności (Grassy & Chavanieu, 2011; Rutkowska et al., 2013). Związki aktywne biologicznie muszą być lipofilowe, żeby były zdolne do przekraczania błon komórkowych, zbudowanych z dwuwarstwy lipidowej. Zbyt mała lipofilowość cząsteczki nie pozwoli na zbliżenie się jej do błony, a zbyt duża sprawi, że cząsteczka najprawdopodobniej utknie w błonie, zamiast ją przekroczyć. Ograniczenia te powodują, że określenie lipofilowości jest konieczne do określenia potencjału nowego leku/pestycydu. Za najbardziej popularny parametr stosowany do tego celu uznaje się logarytm ze współczynnika podziału substancji w układzie n-oktanol/woda (log $P_{o/w}$) (Valkó, 2016). Metoda jego bezpośredniego pomiaru wymaga jednak dysponowania znaczną ilością czystych substancji, precyzyjnym sprzętem pomiarowym, oprócz tego jest żmuda, czasochłonna oraz kosztowna. Jako alternatywne do oceny lipofilowości zostały zaproponowane metody chromatograficzne, w których współczynniki retencji są ściśle zależne od współczynnika podziału Nernsta, opisującego zależność pomiędzy stężeniami substancji w dwóch niemieszających się rozpuszczalnikach (Soares et al., 2022). Równanie typu Collandera wskazuje na istnienie zależności prostoliniowej pomiędzy współczynnikami podziału dla danej substancji w różnych układach

dwufazowych (Collander et al., 1951). Związek pomiędzy współczynnikiem $\log P_{o/w}$ a parametrem chromatograficznym $\log k_w$ (opisującym retencję substancji w czystej wodzie jako fazie ruchomej) ma następującą postać:

$$\log P_{o/w} = a\log k_w + \text{const.} \tag{1}$$

Wyprowadzenie powyższego równania opiera się na przekonaniu, że te same oddziaływania międzycząsteczkowe determinują zachowanie cząsteczki zarówno w układach biologicznych, jak i chromatograficznych. Co więcej, rozwój nauki i techniki umożliwia obecnie wykonywanie pomiarów chromatograficznych w układach imitujących biologiczne.

Modyfikacji klasycznej chromatografii w odwróconym układzie faz dokonuje się albo poprzez zastosowanie specjalnych kolumn chromatograficznych, wypełnionych złożem z immobilizowanym cholesterolem, albuminami, fosfolipidami etc. (Ayouni et al., 2005; Sagandykova et al., 2018) albo poprzez zmianę faz ruchomych na fazy micelarne (Kalyankar et al., 2014; Rambla-Alegre, 2012; Ruiz-Ángel et al., 2009; Tsopelas et al., 2020). Przedstawione właściwości metod chromatograficznych powodują, że podczas poszukiwania substancji biologicznie aktywnych są one uważane za metody *in vitro* (Balouiri et al., 2016; Duijsens et al., 2023; Kraft et al., 2020). Szerszy opis metod chromatograficznych wykorzystanych podczas niniejszego projektu badawczego można znaleźć w sekcji „Metody badawcze".

Istnienie zależności pomiędzy aktywnością biologiczną substancji a jej strukturą cząsteczkową jest znane już od XIX wieku. Metoda Quantitative Structure (Retention)-Activity Relationships (QSARs/QRARs) opiera się na tym założeniu i skupia na poszukiwaniu wieloczynnikowych powiązań pomiędzy właściwościami biologicznymi a parametrami fizykochemicznymi grupy substancji o zbliżonych strukturach (Hansch, 1969, 1993). Niewątpliwą zaletą tej metody są jej zdolności predykcyjne – wyprowadzone modele można z powodzeniem zastosować do przewidywania parametrów biologicznych substancji o podobnej budowie, które nie zostały jeszcze zsyntezowane. Równania matematyczne uzyskiwane z wykorzystaniem QS(R)ARs przedstawiają aktywność biologiczną substancji jako funkcję jej parametrów fizykochemicznych z założeniem, że aktywność biologiczna jest zdefiniowana ilościowo, jako minimalne stężenie substancji wywołujące dany efekt biologiczny (Kaliszan, 1987). Poza lipofilowością do równań wprowadza się również parametry steryczne i elektronowe. Pierwsze z nich opisują właściwości cząsteczki wynikające z jej budowy przestrzennej (cząsteczka nie jest punktem), a drugie – efekt

obecności chmury elektronowej, otaczającej jądra atomowe. Opis metody został przedstawiony w sekcji „Metody badawcze".

Substancje badane w ramach niniejszej pracy doktorskiej zostały zsyntezowane na Uniwersytecie Medycznym w Lublinie (pochodne azoli) oraz w Zakładzie Chemii Organicznej Uniwersytetu Marii Curie-Skłodowskiej (pochodne kwasów karbaminowego i fenoksyoctowego). Przeróżne właściwości lecznicze substancji z grupy triazoli powodują, że warto je rozważać jako potencjalne leki. Związki te charakteryzują się obiecującymi właściwościami przeciwnowotworowymi, przeciwwirusowymi oraz przeciwbólowymi. Część z nich jest zaliczana do kandydatów na środki przeciwnowotworowe, zwłaszcza w leczeniu raka piersi, szyjki macicy, jajnika, płuc, gardła, języka, czy w terapii szpiczaka mnogiego. Dodatkową zaletą badanych pochodnych triazoli jest ich niska toksyczność w stosunku do komórek niezmienionych nowotworowo, m.in. erytrocytów (Janicka et al., 2013; K. Sztanke et al., 2011; M. Sztanke, Rzymowska et al., 2019; M. Sztanke, Sztanke et al., 2019). Szerokie spektrum ich potencjalnych zastosowań wymaga przeprowadzenia badań, opisujących ich drogę po podaniu do ludzkiego organizmu. Pochodne kwasu karbaminowego są uznawane za skuteczne insektycydy. Ze względu na ich zdolność do hamowania acetylocholinoesterazy (oraz innych enzymów z grupy esteraz) chronią ludzi oraz zwierzęta przed chorobami przenoszonymi przez owady. Niektóre pochodne mogą być również transportowane wewnątrz roślin, co czyni je skutecznym środkiem ogólnoustrojowym, zachowującym produkcję rolną (Oliveira et al., 2020; Struger et al., 2016). Z kolei pochodne kwasu fenoksyoctowego zaliczają się do środków chwastobójczych. Po ich wchłonięciu przez nadziemne części rośliny wędrują w stronę korzenia, działając toksycznie zarówno na części nadziemne, jak i podziemne, niszcząc chwasty o rozbudowanym systemie korzeniowym (Czerwiński, 1981).

Badanie nowo zsyntezowanych substancji o potencjalnych właściwościach leczniczych lub ochrony roślin jest ważnym problemem dla współczesnego społeczeństwa. Wybór tematu rozprawy doktorskiej zaowocował przeprowadzeniem istotnych innowacyjnych i multidyscyplinarnych badań z pogranicza kilku dyscyplin naukowych: chemii, farmakologii i matematyki. Dzięki wykorzystaniu metod *in vitro* oraz *in silico*, badania nowych potencjalnych leków/środków ochrony roślin zostały przeprowadzone zgodnie z zasadami zielonej chemii – z ograniczeniem wykorzystywanych odczynników, jak najmniejszym wygenerowaniem odpadów, jak najmniejszym zużyciem rozpuszczalników organicznych oraz przy jak najmniejszych kosztach. Dodatkowo projekt badawczy pozwolił na wykazanie możliwości różnych technik chromatograficznych i obliczeniowych w tak

ważnym aspekcie, jakim jest analiza i przewidywanie właściwości substancji aktywnych biologicznie.

Cel pracy i tezy badawcze

Głównym celem przeprowadzonej pracy badawczej było przewidywanie właściwości farmakokinetycznych oraz aktywności biologicznej nowo zsyntezowanych substancji organicznych z zastosowaniem metody Quantitative Structure (Retention)-Activity Relationships (QRARs/QSARs) oraz różnych technik chromatograficznych. Podczas prowadzonych badań zostały przedstawione następujące cele szczegółowe:

1) przewidywanie *in silico* właściwości fizykochemicznych i biologicznych nowo zsyntezowanych substancji z grupy azoli oraz pochodnych kwasów karbaminowego i fenoksyoctowego;

2) wyznaczenie parametrów retencyjnych log k_w, log k_m, R_M i ich pochodnych oraz ocena tych parametrów jako deskryptorów lipofilowości substancji;

3) wyprowadzenie równań QS(R)ARs i wybór najlepszych z uzyskanych modeli do przewidywania właściwości biologicznych substancji;

4) przewidywanie wpływu lipofilowości substancji na poszczególne parametry farmakokinetyczne oraz toksyczność;

5) wskazanie właściwości fizykochemicznych substancji wpływających na określone parametry farmakokinetyczne oraz toksyczność;

6) uwidocznienie możliwości chromatografii cieczowej do przewidywania właściwości farmakokinetycznych substancji aktywnych biologicznie;

7) wskazanie najlepszych technik chromatograficznych oraz warunków procesu do uzyskania najbardziej wiarygodnych wyników przy wygenerowaniu najmniejszych kosztów;

8) praca zgodnie z zasadami zielonej chemii: sprawdzenie możliwości wielokrotnego wykorzystania płytek chromatograficznych w przypadku technik planarnych.

Zrealizowane badania podkreślają słuszność tezy badawczej – różnorodne techniki chromatograficzne mogą być z powodzeniem stosowane w badaniach przesiewowych do przewidywania właściwości farmakokinetycznych podczas poszukiwania nowych leków/pestycydów z przestrzeganiem zasad zielonej chemii. Przeprowadzone eksperymenty potwierdzają również tezę o możliwościach zastąpienia popularnego parametru lipofilowego log $P_{o/w}$ parametrami chromatograficznymi.

Metody badawcze

W czasie realizacji projektu badawczego zostały wykorzystane różne metody badawcze, zarówno *in silico* (metody obliczeniowe), jak i *in vitro* (metody eksperymentalne). Krótka charakterystyka metod obliczeniowych, chromatograficznych oraz QS(R)ARs została przedstawiona poniżej, a celowość zastosowania ich w prowadzonych badaniach można odnaleźć w części poświęconej uzasadnieniu wyboru tematu. Pełniejsza charakterystyka metod badawczych została przedstawiona w każdym z artykułów cyklu [D1-D4], na którym oparta jest przedstawiana rozprawa doktorska.

1. Metody eksperymentalne – chromatografia cieczowa w odwróconym układzie faz

Chromatografia cieczowa w odwróconym układzie faz RP LC (ang. *Reversed Phase Liquid Chromatography*) to rodzaj chromatografii cieczowej, w którym faza stacjonarna jest mniej polarna od fazy ruchomej (eluentu). Do najczęściej stosowanych faz stacjonarnych należą polarne sorbenty (m.in. żel krzemionkowy) o powierzchni zmodyfikowanej niepolarnymi łańcuchami węglowodorowymi (np.: *n*-oktadecylowymi, *n*-oktylowymi, fenylowymi). Możliwa jest również immobilizacja cząsteczek cholesterolu, protein lub fosfolipidów na powierzchni porowatego żelu krzemionkowego. Takimi fazami są np. sztuczne membrany IAM (ang. *Immobilized Artificial Membranes*) (Barbato et al., 2004; Godard & Grushka, 2011; Nasal et al., 2012; Soukup & Jandera, 2012). Typowa faza ruchoma składa się z mieszaniny wody (bądź buforu) z polarnym rozpuszczalnikiem organicznym, a wzrost jego zawartości w mieszaninie skutkuje wzrostem siły elucyjnej eluentu.

Retencja substancji w danym układzie RP LC zależy od kilku czynników: właściwości fazy stacjonarnej, składu fazy ruchomej, temperatury oraz hydrofobowości próbki (Kaliszan et al., 1999). W przypadku fazy stacjonarnej znaczenie ma zarówno rodzaj niepolarnego złoża, jak i długość modyfikującego go łańcucha, rozmiar porów (powierzchnia właściwa) oraz stopień pokrycia grupami funkcyjnymi. Skład eluentu (zawartość i rodzaj modyfikatora organicznego) decyduje o polarności fazy ruchomej, a w konsekwencji o jej mocy elucyjnej i retencji substancji. Wzrost temperatury powoduje na ogół spadek retencji, ale zbyt wysokie wartości mogą spowodować degradację próbki lub doprowadzić do wrzenia stosowanych rozpuszczalników. Wzajemne oddziaływania pomiędzy fazą stacjonarną, ruchomą i substancją można opisać przy pomocy różnych parametrów. Stosunek czasu spędzonego przez substancję w fazie stacjonarnej do czasu spędzonego w fazie ruchomej

określa się współczynnikiem retencji k. W przypadku zastosowania czystej wody jako eluentu oznaczany jest on jako k_w, a jego logarytm log k_w – zgodnie z decyzją Organizacji Współpracy Gospodarczej i Rozwoju (OECD) – może być wykorzystywany jako deskryptor lipofilowości substancji (Braumann, 1986; OECD Council, 1989).

Micelarna chromatografia cieczowa MLC (ang. *Micellar Liquid Chromatography*) jest jedną z odmian chromatografii cieczowej w odwróconym układzie faz, w której dodatkowym składnikiem polarnej fazy ruchomej jest surfaktant w stężeniu przekraczającym krytyczne stężenie micelizacji cmc (ang. *critical micelle concentration*). Przekroczenie tej wartości powoduje tworzenie się w roztworze miceli oraz modyfikację powierzchni fazy stacjonarnej przez monomery surfaktantu na skutek hydrofobowych oddziaływań pomiędzy niepolarnym ogonem surfaktantu a łańcuchami węglowodorowymi (Rambla-Alegre, 2012; Ruiz-Ángel et al., 2009). Faza ruchoma może być dwuskładnikowa, składająca się tylko z wody (buforu) i surfaktantu, ale znacznie częściej stosowane są fazy z dodatkiem modyfikatora organicznego. Do najczęściej stosowanych rozpuszczalników należy zaliczyć krótkołańcuchowe alkohole alifatyczne (np. *n*-propanol, *n*-butanol) (Dorsey et al., 1983), ale za skuteczne modyfikatory są również uważane kwasy karboksylowe, jak i rozpuszczalniki organiczne, m.in. tetrahydrofuran, acetonitryl, czy dioksan (Kord & Khaledi, 1993; López Grío et al., 1999). Dodatek modyfikatora zwiększa siłę elucyjną fazy ruchomej, poprawia selektywność rozdziału oraz zmniejsza asymetryczność otrzymywanych pików (Dorsey et al., 1983). Obecnie prowadzone są badania mające na celu eliminację rozpuszczalnika organicznego z fazy ruchomej. Dobra selektywność oraz siła elucyjna powinny zostać utrzymane dzięki dodatkowi drugiego surfaktantu do micelarnej fazy ruchomej (Bahgat et al., 2022; Peris-Garcia et al. 2017).

Ze względu na budowę chemiczną surfaktanty można podzielić na jonowe (kationowe i anionowe), niejonowe i amfoteryczne. Najpowszechniej stosowanymi surfaktantami jonowymi są dodecylosiarczan sodu SDS (surfaktant anionowy) oraz bromek cetylotrimetyloamoniowy CTAB (surfaktant kationowy). Szczególnym przypadkiem jest zastosowanie obojętnego surfaktantu Brij 35 (eter laurylowy glikolu polioksyetylenowego (23)). W takim przypadku technika przybiera nazwę BMC (ang. *Biopartioning Micellar Chromatography*), a warunki obecne w układzie odpowiadają warunkom istniejącym przy przekraczaniu barier biologicznych w żywych organizmach (Tsopelas et al., 2020).

Obecność surfaktantu w roztworze wpływa na oddziaływania międzycząsteczkowe, a w konsekwencji na mechanizm retencji substancji (Kalyankar et al., 2014). Wprowadzony do układu analit oddziałuje zarówno z grupami hydrofilowymi cząsteczek surfaktantu obecnymi w fazie ruchomej, ze zmodyfikowaną monomerami powierzchnią fazy stacjonarnej oraz penetruje powstałe micele. Wymienione oddziaływania skutkują powstaniem trzech stanów równowagi, opisujących asocjację (lub wiązanie) analitu w miceli (K_{AM}), asocjację analitu z powierzchnią fazy stacjonarnej zmodyfikowanej monomerami surfaktantu (K_{WS}) i bezpośredni transfer analitu z miceli na powierzchnię fazy stacjonarnej (K_{MS}) (Armstrong & Nome, 1981; Arunyanart & Cline Love, 1984). Wpływ stężenia surfaktantu obecnego w fazie ruchomej na retencję substancji pozwolił na wprowadzenie parametru k_m, jako współczynnika retencji analitu przy zerowym stężeniu „wolnego" surfaktantu w fazie objętościowej, tj. przy stężeniu surfaktantu równym cmc (Foley, 1990). Logarytm współczynnika retencji w układach micelarnych log k_m jest analogiczny do parametru log k_w obecnego w klasycznej chromatografii w odwróconym układzie faz. Podczas analizy substancji aktywnych biologicznie parametry chromatografii micelarnej log k_m oraz K_{AM} są stosowane do opisu lipofilowości badanych związków.

W przedstawianym projekcie badawczym zostały przeprowadzone eksperymenty, wykorzystujące chromatografię cieczową, z zastosowaniem zarówno techniki kolumnowej, jak i planarnej. W publikacji D1 zaprezentowano analizę dziewiętnastu substancji z grupy azoli o właściwościach przeciwnowotworowych. W chromatografii RP LC wykorzystano kolumny chromatograficzne IAM oraz RP-18e, a jako polarne eluenty zastosowano buforowane (pH wynoszące 7,4) rozwory acetonitrylu. Uzyskane wyniki umożliwiły wyliczenie chromatograficznych parametrów lipofilowości log k_w. Retencja wspomnianych substancji została również zmierzona techniką BMC. Pomiary zostały przeprowadzone z użyciem kolumny chromatograficznej RP-18e, podczas gdy buforowane (pH wynoszące 7,4) roztwory Brij 35 z 7-procentowym dodatkiem izopropanolu (v/v) zostały wykorzystane jako polarna faza ruchoma. Na podstawie zebranych danych wyliczono ekstrapolowane parametry log k_m oraz log $k_{0.1}$ (dla fazy ruchomej, w której stężenie Brij 35 wynosiło 0,1 mol L^{-1}). W publikacjach D2-D4 zaprezentowano wykorzystanie techniki MLC (z anionowym surfaktantem SDS) do analizy potencjalnych pestycydów [D2] i substancji leczniczych o szerokim spektrum zastosowania (przeciwnowotworowym, przeciwbólowym, przeciwwirusowym) [D3, D4]. Dokładny opis warunków doświadczalnych podczas badań lipofilowości pochodnych kwasów karbaminowego i fenoksyoctowego został przedstawiony

w pracy D2. Zastosowanie buforowanego roztworu SDS (pH równe 7,4) z 20-procentowym dodatkiem acetonitrylu (v/v) jako polarnego eluentu oraz niepolarnej kolumny chromatograficznej RP-8e pozwoliło na wyliczenie parametrów $\log k_m$ i $\log K_{AM}$. W publikacji D3 wykorzystano chromatografię planarną (Ciura et al., 2017; Komsta et al., 2013) – zmierzono retencję (wartości R_M) 14. substancji z grupy azoli z użyciem płytek TLC RP-CN i buforowanego roztworu SDS (pH równe 7,4) z 20-procentowym dodatkiem tetrahydrofuranu (v/v) jako modyfikatora organicznego. W pracy D4 badano 65 substancji z grupy azoli techniką kolumnową. Buforowany roztwór SDS (pH równe 7,4) z 7-procentowym dodatkiem izopropanolu (v/v) stosowano jako fazę ruchomą na kolumnie RP-18e, a jako parametry lipofilowości substancji rozważano wartości $\log (k_m/K_{AM})$.

2. Metody obliczeniowe (*in silico*)

Parametry fizykochemiczne, farmakokinetyczne oraz toksyczność badanych substancji zostały wyliczone na podstawie ich struktur z użyciem oprogramowania ACD/Percepta. Dokładne wartości można znaleźć w tabelach zamieszczonych w publikacjach D1-D4. Zastosowanie metody *in silico* pozwoliło na wyliczenie następujących parametrów:

- fizykochemicznych: masy molowej MW [D1-D4], polaryzowalności α [D1, D3, D4], topologicznego pola powierzchni polarnej $TPSA$ [D1, D2, D4], liczby akceptorów HBA i donorów HBD wiązania wodorowego [D2, D4], liczby wiązań rotacyjnych NRB [D2, D4], parachory P [D4]; współczynnika podziału w układzie n-oktanol/woda $\log P_{o/w}$ [D1, D2, D4],
- farmakokinetycznych: $\log BB$ [D1-D4], $\log K_{HSA}$, $f_{u,brain}$ [D1-D3], $\log K_p$ [D1, D2], $Caco-2$ [D1], V_d, $f_{u,plasma}$, $\log K_{sc}$, $\log K_{w/cell}$, $\log P_{w/HSA}$, $\log P_{w/pc}$ [D2];
- toksyczności: LD_{50} [D2].

Dla wszystkich wymienionych powyżej parametrów farmakokinetycznych oraz toksyczności zostały wyprowadzone równania QS(R)ARs.

W publikacji D1 metody *in silico* zostały także wykorzystane do wyliczenia ryzyka działań niepożądanych badanych substancji. Potrzebne obliczenia wykonano z użyciem oprogramowania OSIRIS, a uzyskane dane zostały zebrane w materiałach uzupełniających do pracy D1.

3. Quantitative Structure (Retention)-Acitivity Relationships

Metoda Quatitative Retention-Activity Relationships (QRARs) oraz Quatitative Structure-Activity Relationships (QSARs) opiera się na powiązaniu aktywności biologicznej substancji z retencją chromatograficzną oraz/lub z różnymi parametrami fizykochemicznymi charakteryzującymi te substancje. Podstawy metody można zaprezentować w postaci poniższego modelu matematycznego (Hansch, 1993; Héberger, 2007; Kaliszan, 1987):

$Aktywność = f($właściwości lipofilowe, elektronowe i steryczne$) = aA + bB + cC + ... + const.$ (2)

Istnieje możliwość przewidywania właściwości biologicznych substancji tylko na podstawie ich współczynników retencji (QRARs). W tym celu dla badanej grupy substancji lipofilowość musi być decydującym czynnikiem wpływającym na ich aktywność biologiczną (Grassy & Chavanieu, 2011). Konieczne jest także występowanie dobrej korelacji (liniowej lub parabolicznej) pomiędzy parametrami retencyjnymi i biologicznymi (Breyer et al., 1991; Quiñones-Torrelo et al., 2005). W przypadku braku takiej korelacji wprowadzane są do równania kolejne zmienne niezależne, tworząc równanie wielokrotnej regresji linowej MLR (ang. *multiple linear regression*) (Eriksson et al., 2003). Do najczęściej dodawanych deskryptorów opisujących właściwości steryczne substancji należą: masa molowa, objętość molowa, polaryzowalność czy parachora. Parametr opisujący liczbę wiązań rotacyjnych informuje o elastyczności cząsteczki jako jej właściwości sterycznej (Liu et al., 2001; Pourbasheer et al., 2010). Topologiczne pole powierzchni polarnej oraz liczba donorów i akceptorów wiązania wodorowego stanowią grupę deskryptorów elektronowych (Abraham et al., 1994; Ertl et al., 2000; Kelder et al., 1999; Van De Waterbeemd et al., 1998). Popularnym parametrem wyrażającym lipofilowość cząsteczki jest log $P_{o/w}$. Trudności, jakie sprawia dokładny pomiar tego deskryptora, powodują jednak, że najczęściej wybieranymi parametrami lipofilowymi przy wyprowadzaniu równań QSARs są chromatograficzne parametry retencyjne (Valkó, 2016).

Jednym z założeń metody QSARs jest możliwość przewidywania właściwości farmakokinetycznych nowych, niebadanych wcześniej związków. Wyprowadzenie równania w oparciu o parametry uzyskane dla grupy związków chemicznych o strukturze zbliżonej do nowo analizowanych pozwala na przewidywanie ich aktywności z dobrym prawdopodobieństwem. Opisany sposób ułatwia poszukiwanie nowych substancji o pożądanych cechach. W przypadku wyprowadzania nowych zależności należy pamiętać o wykorzystaniu wiarygodnych i dokładnych danych, odpowiednim wyborze wprowadzanych deskryptorów, a także o walidacji uzyskanego modelu (Eriksson et al., 2003; OECD

Council, 2007). Parametry fizykochemiczne wykorzystywane w równaniu muszą różnicować badane substancje, co oznacza, że nie mogą być ze sobą wzajemnie powiązane i zależne (Gramatica, 2012). Sprawdzenie wzajemnych powiązań wprowadzanych parametrów (zmiennych niezależnych) jest możliwe m.in. dzięki zastosowaniu analizy skupień (np. dendrogramów) czy analizy głównych składowych PCA (ang. *Principal Component Analysis*). Pomocny jest w tym przypadku także współczynnik wariancji inflacji *VIF* (ang. *variance inflation factor*). Wyliczenie współczynnika determinacji R^2 pozwala na ocenę dopasowania uzyskanego modelu do danych wyjściowych. Korelacje, w których otrzymano wartości R^2 powyżej 0,8 uznaje się za dobre. Rzadziej spotykanymi współczynnikami determinacji są $R^2_{adj.}$ oraz $R^2_{pred.}$ – pierwszy z nich używany jest do porównania między sobą dopasowania modeli regresji, które zawierają różną liczbę zmiennych niezależnych, a drugi obrazuje wiarygodność prognoz tworzonych przez uzyskany model (How to Interpret Adjusted R-Squared and Predicted R-Squared in Regression Analysis, n.d.). Do często używanych parametrów statystycznych można ponadto zaklasyfikować prawdopodobieństwo testowe *p* oraz odchylenie standardowe *sd*. Wyprowadzone równania QSARs poddawane są walidacji krzyżowej oraz sprawdzeniu korelacji pomiędzy wartością wyliczoną i rzeczywistą. Wykorzystanie walidacji leave-out pozwala na sprawdzenie dopasowania wyprowadzonego modelu poprzez odrzucenie jednej lub więcej zmiennych ze zbioru danych i porównaniu parametrów statystycznych uzyskanych dla zależności wyprowadzonych dla pełnego i niepełnego zbioru. Dziedziny zastosowania modelu AD (ang. *applicability domain*) (Roy et al., 2015; Sahigara et al., 2012), w których powstały model powinien umożliwiać prognozowanie z dobrym prawdopodobieństwem, obrazuje się wykresami Williamsa. Wspomniane wykresy umożliwiają wyodrębnienie wartości odstających, tak samo jak wykorzystanie korelacji pomiędzy wartościami obliczonymi na podstawie wyprowadzonych równań a wartościami rzeczywistymi. Jakościowy oraz ilościowy wpływ poszczególnych parametrów fizykochemicznych na dane właściwości farmakokinetyczne często przedstawia się na wykresach standaryzowanych współczynników.

Podczas niniejszego projektu badawczego dla wszystkich badanych grup związków wyprowadzono równania łączące ich aktywność biologiczną z parametrami retencji (jako deskryptorami lipofilowości) oraz deskryptorami właściwości sterycznych i/lub elektronowych [D1-D4]. Parametry statystyczne wykorzystane do oceny równań zostały szerszej scharakteryzowane w każdej z publikacji cyklu. W wyprowadzonych równaniach QSARs zmiennymi niezależnymi, obok lipofilowości chromatograficznej, są masa molowa

[D1, D3, D4], parachora [D4], liczba wiązań rotacyjnych [D2, D4] lub polaryzowalność [D1, D3] jako parametry steryczne, oraz liczba donorów/akceptorów wiązania wodorowego [D2, D4] i topologiczne pole powierzchni polarnej [D1, D4] jako parametry elektronowe. Walidacja powstałych zależności QSARs została zobrazowana wykresami standaryzowanych współczynników, korelacji uzyskanych pomiędzy wartościami wyliczonymi a rzeczywistymi oraz wykresami Williamsa.

Zwięzłe omówienie wyników

Badania zaprezentowane w cyklu publikacji w pełni realizują cele szczegółowe projektu badawczego. Cel pierwszy został osiągnięty poprzez wyznaczenie *in silico* (ACD/Percepta) parametrów fizykochemicznych (log $P_{o/w}$, *MW*, *α*, *P*, *NRB*, *HBD*, *HBA*, *TPSA*), farmakokinetycznych (log *BB*, log K_{HSA}, log $P_{w/HSA}$, log K_p, *Caco-2*, V_d, $f_{u,brain}$, $f_{u,plasma}$, log K_{sc}, log $K_{w/cell}$ i log $P_{w/pc}$) oraz parametru opisującego toksyczność (LD_{50}) badanych substancji. Podczas analizy potencjalnych leków wyznaczono parametry opisujące przenikanie przez bariery biologiczne, wiązanie się substancji z białkami surowicy krwi oraz wolne frakcje w mózgu i osoczu [D1, D3, D4]. Badania potencjalnych pestycydów wymagały wyznaczenia dodatkowych deskryptorów, opisujących wnikanie substancji z wody do komórki roślinnej oraz toksyczność. Wszystkie dane uzyskane metodą *in silico* zostały zebrane i zaprezentowane w cyklu artykułów: Tabela 1. w publikacji D1, Tabela 2. w publikacji D2, Tabela 1. w publikacji D3 oraz Tabela 3. w publikacji D4. Przedstawione wartości liczbowe nie pozwalają jednak na wnioskowanie o wpływie poszczególnych parametrów fizykochemicznych na dany parametr farmakokinetyczny lub toksyczny. Dokładna analiza powiązania aktywności biologicznej substancji z jej strukturą była możliwa dopiero po wykonaniu kolejnych kroków badawczych i wyprowadzeniu równań QS(R)ARs. Wartości parametrów farmakokinetycznych obliczonych podczas realizacji pierwszego celu szczegółowego zostały później wykorzystane podczas realizacji celu trzeciego, czyli porównania danych uzyskanych *in silico* z danymi wyliczonymi na podstawie wyprowadzonych równań. Pozwoliło to na ocenę wiarygodności wyprowadzonych modeli QSARs.

Wyznaczenie chromatograficznych parametrów lipofilowości: log k_w [D1], log k_m [D1, D2], log K_{AM} [D2], log (k_m/K_{AM}) [D4] i R_M [D3] oraz ich porównanie z *in silico* wartościami log $P_{o/w}$ pozwoliło na realizację drugiego celu pracy doktorskiej. W publikacjach D2 i D4 zależności pomiędzy chromatograficznymi parametrami lipofilowości oraz współczynnikami podziału log $P_{o/w}$ dla badanych związków zostały zaprezentowane w sposób graficzny. Rysunek 1. w pracy D2 poświęconej pestycydom obrazuje bardzo dobrą zależność prostoliniową pomiędzy parametrami log k_m i log $P_{o/w}$ (R = 0,9566 dla pochodnych kwasu karbaminowego i R = 0,9985 dla pochodnych kwasu fenoksyoctowego) oraz log K_{AM} i log $P_{o/w}$ (R = 0,8978 dla pochodnych kwasu karbaminowego i R = 0,9869 dla pochodnych kwasu fenoksyoctowego). Dodatkowo wyprowadzono równanie opisujące zależność

pomiędzy dwa wspomnianymi parametrami lipofilowymi - $\log k_m$ vs $\log K_{AM}$. Bardzo dobrą korelację pomiędzy deskryptorami potwierdza współczynnik $R = 0{,}9861$. Zależności pomiędzy wymienionymi parametrami potwierdzają możliwość stosowania obu micelarnych parametrów chromatograficznych jako deskryptorów lipofilowości. Bardzo dobra korelacja pomiędzy wyliczonymi *in silico* współczynnikami $\log P_{o/w}$ a parametrami micelarnymi $\log (k_m/K_{AM})$ została przedstawiona w publikacji D4 na Rysunku 2. Współczynnik determinacji $R^2 = 0{,}7980$ uzasadnił zastosowanie parametru $\log (k_m/K_{AM})$ jako deskryptora lipofilowości substancji w dalszej części prowadzonych badań. Parametr R_M przedstawiony w pracy D3 to współczynnik retencji stosowany w chromatografii planarnej, analogiczny do $\log k$, powszechnie akceptowanego jako chromatograficzny parametr lipofilowy.

Analiza uzyskanych danych pozwala na stwierdzenie, że parametry chromatograficzne $\log k_m$ i $\log K_{AM}$ spełniają swoją rolę przy opisie lipofilowości badanych pestycydów, przy czym nieznacznie lepsze wyniki otrzymuje się dla deskryptora $\log k_m$ [D2]. Oznacza to, że układ chromatograficzny, w którym fazę ruchomą stanowi roztwór SDS z dodatkiem acetonitrylu, można z powodzeniem stosować w badaniach lipofilowości pochodnych kwasów karbaminowego i fenoksyoctowego. Publikacja D4 wskazuje z kolei na fakt, że do wyznaczania lipofilowości badanych potencjalnych leków o szerokim spektrum stosowania najlepiej zastosować metodę MLC z dodatkiem surfaktantu SDS oraz izopropanolu jako modyfikatora organicznego.

Celem trzecim poniższego opracowania było wyprowadzenie równań QRARs i QSARs, czyli matematycznych zależności łączących aktywność i toksyczność substancji z jej właściwościami fizykochemicznymi (lipofilowymi, strukturalnymi, elektronowymi). W przypadku metody QRARs znajomość lipofilowości substancji (parametrów chromatograficznych wyznaczonych *in vitro*) jest wystarczająca do przewidywania jej bioaktywności. W prowadzonych badaniach było to możliwe dla niektórych deskryptorów farmakokinetycznych ($\log K_{sc}$, $\log P_{w/HSA}$, V_d, $\log P_{w/pc}$, $f_{u,plasma}$ i $f_{u,brain}$) badanych pestycydów. Otrzymane wyniki [D2] wskazują, że lipofilowość jest najważniejszą cechą determinującą wspomniane właściwości analizowanych środków ochrony roślin. Dla większości rozważanych parametrów biologicznych konieczne jest jednak poszukiwanie równań wieloparametrowych. W tym celu stosuje się metodę MLR. W rozważanych modelach QSARs jako zmienne niezależne rozważane były wymienione wcześniej chromatograficzne parametry lipofilowości (parametry *in vitro*) oraz wyznaczone *in silico* deskryptory strukturalne i/lub elektronowe badanych substancji. Wyboru zmiennych niezależnych

dokonuje się w oparciu o analizę statystyczną – jak zostało wspomniane w opisie metody, poszczególne zmienne muszą różnicować badane substancje i spełniać wymóg ortogonalności. W przedstawianych badaniach wyboru zmiennych niezależnych dokonano na podstawie parametru *VIF* [D1-D3] i analizy skupień (dendrogram w publikacji D4). Wszystkie wyprowadzone zależności zostały poddane walidacji oraz analizie statystycznej, aby ocenić ich poprawność, wiarygodność i możliwości predykcyjne.

Wyprowadzone modele QRARs łączą strukturę substancji tylko z ich właściwościami lipofilowymi. W takim wypadku oceny powstałych modeli dokonuje się na podstawie analizy poprawności dopasowania uzyskanego modelu do wprowadzonych danych (współczynnik korelacji *R*). Ten sposób został zastosowany podczas badań nad potencjalnymi pestycydami [D2]. Analiza statystyczna modeli QRARs przedstawia wartości współczynników korelacji *R* pomiędzy parametrami farmakokinetycznymi a chromatograficznymi deskryptorami lipofilowości. Uzyskane wartości znajdują się w przedziale od 0,9375 do 0,9829 dla powstałych zależności liniowych (parametry farmakokinetyczne $\log K_{sc}$ i $\log P_{w/HSA}$) oraz od 0,9290 do 0,9965 dla zależności parabolicznych (parametry V_d, $\log P_{w/pc}$, $f_{u,plasma}$, $f_{u,brain}$). Wszystkie powyższe wartości przekraczają graniczną wartość 0,8, pozwalając na uznanie uzyskanych modeli QRARs za bardzo dobre. Dla pięciu z badanych parametrów ($\log P_{w/pc}$, $f_{u,plasma}$, $f_{u,brain}$, $\log K_{sc}$ i $\log P_{w/HSA}$) wyższe wartości współczynnika *R* uzyskuje się dla korelacji z parametrami $\log k_m$. W przypadku objętości dystrybucji V_d nieznacznie lepsze dopasowanie uzyskuje się podczas zastosowania współczynnika $\log K_{AM}$. Porównanie uzyskanych wyników z danymi otrzymanymi podczas realizacji celu drugiego (ocena parametrów chromatograficznych jako deskryptorów lipofilowości) pozwala wnioskować, że dla badanych pestycydów w przypadku wyprowadzania modeli QRARs parametr $\log k_m$ jest lepszym deskryptorem lipofilowości niż $\log K_{AM}$.

Modele QSARs zostały poddane bardziej rozbudowanej analizie statystycznej. Dla wszystkich wyprowadzonych równań obliczono współczynniki korelacji *R* [D2, D3] lub determinacji R^2 [D1, D4] pomiędzy wartościami badanych parametrów wyliczonych na podstawie wyprowadzonych równań a obliczonych *in silico*. Dla badanych pestycydów wartości współczynników *R* mieściły się w zakresie od 0,8722 do 0,9893 [D2]. Równania opisujące parametry farmakokinetyczne substancji leczniczych są zamieszczone w publikacjach D1, D3 i D4. Dla wszystkich analizowanych parametrów otrzymano bardzo dobre korelacje. Wyliczone współczynniki *R* znajdowały się w przedziale od 0,9178 do 0,9512 [D3], a współczynniki R^2 od 0,8799 do 0,9723 [D1] oraz od 0,8508 do 0,9260 [D4].

Ponownie wszystkie uzyskane wartości są wyższe od 0,8. Dla wyprowadzonych równań QSARs zostały także wyliczone współczynniki $R^2_{adj.}$ ($R_{adj.}$) [D1-D4] oraz $R^2_{pred.}$ [D4]. Współczynniki $R^2_{pred.}$ opisują zdolności predykcyjne otrzymanych modeli. W zaprezentowanych badaniach jego wartości mieściły się w granicach pomiędzy 0,8297 i 0,9150, potwierdzając bardzo dobre zdolności predykcyjne wyprowadzonych równań. Współczynniki $R^2_{adj.}$ ($R_{adj.}$) uławiają porównanie pomiędzy sobą modeli, opisujących ten sam parametr farmakokinetyczny, a różniących się liczbą wprowadzonych zmiennych niezależnych. Wyższe wartości świadczą o lepszym dopasowaniu danych do wybranego modelu. Możliwości zastosowania tego parametru statystycznego są najlepiej widoczne w publikacji D4 (Tabela 4.), gdzie modele wyprowadzone dla parametru $\log BB$ zostały wyprowadzone z użyciem zarówno trzech, jak i czterech zmiennych niezależnych. Analiza statystyczna wykazała, że mniejsza liczba zastosowanych zmiennych niezależnych powoduje uzyskanie lepszych wyników.

Parametry fizykochemiczne wprowadzane do równania muszą różnicować dane substancje. Ocenę wzajemnej zależności stosowanych zmiennych umożliwia współczynnik *VIF* – w przypadku uzyskania wartości wyższej od 5,0 uzyskany model ocenia się jako niewiarygodny. W prowadzonych badaniach wartości tego parametru znajdowały się zakresie niższym niż 3,6 dla badanych pestycydów [D2] oraz poniżej 4,8 dla badanych leków [D1, D3, D4].

Porównanie różnych parametrów statystycznych przed i po przeprowadzeniu walidacji krzyżowej zostało zaprezentowane w pracach D1, D2 i D4. Wartości współczynników determinacji R^2 znajdują się w Tabelach 3. i 4. w publikacji D1 oraz w Tabeli 4. w publikacji D4. Łatwo można dostrzec, że dla prawie wszystkich wyprowadzonych równań wartość współczynników R^2 otrzymanych przed walidacją oraz po niej nie uległa zmianie. Taka obserwacja potwierdza dobre dopasowanie modeli QSARs dla badanych leków. Analogiczne porównanie wykonano dla przewidywanego błędu resztowego sumy kwadratów *PRESS* (ang. *predicted residual error sum of squares*) [D1, D2, D4] i średniego błędu kwadratowego *MSE* (ang. *mean square error*) [D1, D2]. Wartości tych parametrów zależne są od wartości parametrów farmakokinetycznych, w związku z tym nie istnieje wartość graniczna, od której uzyskany model można uznać za dobry. Uzyskanie takich samych lub zbliżonych wartości przed i po wykonaniu walidacji pozwala uznać wyprowadzony model QSARs za dobry i wiarygodny (Clementi et al., 2001). Analiza dziedzin zastosowania modeli, zobrazowana wykresami Williamsa [D1-D4], wykazuje, że w żadnych z nich nie zaobserwowano wartości

odstających, po raz kolejny potwierdzając wiarygodność wyprowadzonych równań.

Porównanie parametrów statystycznych umożliwiło także wybór najlepszych układów chromatograficznych do wyznaczania deskryptorów farmakokinetycznych opisujących zdolność badanych leków do przenikania przez barierę krew-mózg oraz ich transportu w organizmie. Zastosowanie surfaktantu Brij 35 w chromatografii micelarnej (BMC) pozwala na wiarygodne przewidywanie parametru $\log BB$, nawet po wykonaniu pomiarów w tylko jednej fazie ruchomej [D1]. W przypadku parametrów $\log K_{HSA}$ oraz $f_{u,brain}$ najlepszym układem chromatograficznym okazał się opisany w publikacji D2, z zastosowaniem płytek TLC RP-CN jako fazy stacjonarnej oraz buforowanego roztworu SDS z dodatkiem tetrahydrofuranu jako fazy ruchomej.

Ocena wpływu lipofilowości na aktywność biologiczną substancji jest tożsama ze zrealizowaniem czwartego celu badawczego. Lipofilowość, jako czynnik decydujący w znacznym stopniu o aktywności biologicznej substancji, został włączony do wszystkich wyprowadzonych równań. Niezależnie od stosowanej techniki chromatograficznej i rodzaju parametru lipofilowości ($\log k_w$, $\log k_m$, $\log K_{AM}$, $\log (k_m/K_{AM})$, R_M) jakościowy wpływ lipofilowości substancji na dany parametr farmakokinetyczny jest analogiczny. W badanych zakresach w przypadku deskryptorów opisujących przenikanie przez bariery biologiczne, tj. barierę krew-mózg ($\log BB$) [D1-D4], przez skórę ($\log K_p$ [D1, D2], $\log K_{sc}$ [D2]), wchłanianie zwrotne w jelitach (*Caco-2*) [D1], wzrost lipofilowości substancji powoduje wzrost wartości parametrów farmakokinetycznych. Parametry opisujące wiązanie substancji biologicznie aktywnej z albuminą ($\log P_{w/HSA}$ [D2], $\log K_{HSA}$ [D1-D3]) również rosną wraz ze wzrostem lipofilowości. Jako naturalna konsekwencja, wartości parametrów opisujących frakcje niezwiązane w mózgu i osoczu ($f_{u,brain}$ [D1-D3], $f_{u,plasma}$ [D2]) maleją, gdy lipofilowość substancji rośnie. Wzrost lipofilowości wpływa również dodatnio na objętość dystrybucji (V_d), przenikanie substancji do komórek roślinnych ($\log K_{w/cell}$) i powoduje wzrost parametru opisującego podział substancji pomiędzy wodę a nabłonek (kutykulę) rośliny ($\log P_{w/pc}$) [D2]. Zależność odwrotnie proporcjonalną obserwuje się pomiędzy lipofilowością a dawką śmiertelną LD_{50} (wyznaczanej dla potencjalnych pestycydów) – wzrost lipofilowości powoduje spadek dawki śmiertelnej dla danego związku, a więc wzrost toksyczności substancji bioaktywnej [D2].

Piąty cel szczegółowy polegał na analizie wpływu parametrów fizykochemicznych na dany parametr farmakokinetyczny. Graficzne przedstawienie danych z użyciem wykresów standaryzowanych współczynników ułatwiło jego realizację. Interpretacja danych wskazuje,

że przenikanie substancji przez barierę krew-mózg (log BB) rośnie ze wzrostem polaryzowalności substancji [D1, D3] i parachory [D4]. Z kolei wzrost topologicznego pola powierzchni polarnej [D1], liczby donorów wiązania wodorowego [D2] i wiązań rotacyjnych [D2, D4] oraz masy molowej substancji [D3] powoduje spadek przenikania substancji przez wspomnianą barierę. Ciekawym przypadkiem jest liczba akceptorów wiązania wodorowego. Zgodnie z uzyskanymi wynikami może ona powodować zarówno wzrost [D2], jak i spadek [D4] wartości parametru log BB. Należy zwrócić uwagę na fakt, że wpływ parametru HBA na właściwości farmakokinetyczne substancji wykazany w publikacji D2 jest niewielki. Uznaje się, że zależność większości parametrów farmakokinetycznych od właściwości fizykochemicznych substancji posiada charakter paraboliczny – po przekroczeniu pewnej granicznej wartości (unikalnej dla każdego parametru fizykochemicznego i farmakokinetycznego) tendencja wpływu właściwości substancji na wartość jej deskryptora farmakokinetycznego ulega odwróceniu [D4]. Najprawdopodobniej badane substancje znajdują się w obszarze „przejściowym" i w przypadku pestycydów liczba akceptorów wiązania wodorowego HBA powoduje wzrost ich przenikania przez barierę krew-mózg, natomiast w przypadku leków – spadek przenikania substancji leczniczych przez wspomnianą barierę. Wartości deskryptora log K_{HSA} rosną ze wzrostem polaryzowalności, masy molowej [D1, D3], liczby donorów wiązania wodorowego i wiązań rotacyjnych [D2]. Ze wszystkich przeanalizowanych parametrów fizykochemicznych tylko wzrost liczby akceptorów wiązania wodorowego powoduje spadek wiązania substancji z białkami surowicy krwi [D2]. Wzrost topologicznego pola powierzchni polarnej powoduje wzrost niezwiązanej frakcji w mózgu $f_{u,brain}$ [D1]. Zarówno wzrost masy molowej, jak i polaryzowalności powodują zmniejszenie wolnej frakcji badanych substancji w mózgu [D1, D3]. Wartości parametru log K_p rosną ze wzrostem polaryzowalności, masy molowej [D1] i liczby wiązań rotacyjnych [D2], natomiast maleją ze wzrostem liczby akceptorów i donorów wiązania wodorowego [D2] oraz topologicznego pola powierzchni polarnej [D1]. Analogiczny wpływ można również dostrzec na parametr $Caco-2$ – jego wartości maleją ze wzrostem masy molowej i topologicznego pola powierzchni polarnej [D1]. Wzrost wartości parametru log $K_{w/cell}$ jest powiązany z malejącą liczbą akceptorów i donorów wiązania wodorowego, natomiast przenikanie substancji do komórek roślinnych wzrasta z liczbą wiązań rotacyjnych [D2]. Inne zależności obserwuje się dla parametru toksyczności LD_{50}, którego wzrost wartości następuje ze wzrostem liczby donorów wiązania wodorowego, a spadek (czyli wzrost toksyczności substancji) łączy się ze

wzrostem liczby akceptorów wiązania wodorowego i wiązań rotacyjnych w cząsteczce substancji [D2].

Przeprowadzone badania w pełni realizują cel szósty, obrazując potencjał metod chromatograficznych do opisu właściwości lipofilowych substancji oraz ukazując przewagę chromatograficznych parametrów lipofilowych log k_w, log k_m (R_M), log K_{AM} nad tradycyjnym parametrem log $P_{o/w}$. Wykorzystanie chromatografii jako techniki *in vitro* umożliwia sprawne oraz wiarygodne określenie lipofilowości substancji bioaktywnych. Wybór najlepszych technik chromatograficznych (cel siódmy) jest zależny od liczby analizowanych substancji i typu przeprowadzanych pomiarów. W przypadku wstępnych badań przesiewowych doskonale sprawdzają się techniki cienkowarstwowe [D3]. Pomiary parametrów retencyjnych można wykonać dla nawet kilkunastu substancji jednocześnie, przyspieszając proces badawczy, równocześnie zużywając niewielkie ilości odczynników i generując niewielkie ilości odpadów. Analiza statystyczna równań QSARs wyprowadzonych na podstawie danych otrzymanych w ten sposób jednoznacznie wskazuje, że uzyskane w taki sposób wyniki są w pełni wiarygodne. Dodatkowo, przy przeprowadzaniu wstępnych badań istnieje możliwość regeneracji rozwiniętych (zużytych) płytek i ich ponownego wykorzystania, co znacząco wpływa na obniżenie kosztów takich badań. Przeprowadzenie sprawnych pomiarów chromatograficznych w optymalnych warunkach (tj. najbardziej zbliżonych do obecnych w żywym organizmie) jest również możliwe dzięki wykorzystaniu chromatografii BMC [D1]. Zastosowanie obojętnego surfaktantu Brij 35 pozwala na wykonanie pomiarów w tylko jednej fazie ruchomej w celu uzyskania wiarygodnych chromatograficznych parametrów lipofilowości substancji. Poprawność takiego rozwiązania została sprawdzona z wykorzystaniem analizy statystycznej. Zastosowanie tej drogi pokrywa się z ideą celu ósmego, dotyczącego pracy zgodnie z zasadami zielonej chemii. Pozostałe techniki chromatograficzne wykorzystane w czasie pomiarów eksperymentalnych również skutkują wiarygodnymi wynikami, ale trwają znacznie dłużej i wymagają większych nakładów finansowych.

Podsumowanie

Przeprowadzony projekt badawczy umożliwił ocenę możliwości zastosowania nowo syntezowanych substancji jako potencjalne leki lub pestycydy poprzez zrealizowanie wszystkich celów szczegółowych wskazanych w niniejszej pracy. Badania eksperymentalne, wykonane techniką chromatografii cieczowej kolumnowej i planarnej, doprowadziły do oznaczenia lipofilowości badanych związków z wykorzystaniem parametrów chromatograficznych: $\log k_w$ [D1], $\log k_m$ [D1, D2], $\log K_{AM}$, [D2] $\log (k_m/K_{AM})$ [D4] oraz R_M [D3]. Układy chromatograficzne użyte podczas wszystkich przeprowadzanych eksperymentów tworzyły środowisko imitujące biologiczne, pozwalając na odtworzenie procesów zachodzących w komórkach żywych organizmów (badania *in vitro*).

Połączenie wyników uzyskanych dzięki przeprowadzonym eksperymentom z danymi wyliczonymi metodami obliczeniowymi (*in silico*) umożliwiło wyprowadzenie równań typu Quantitative Structure (Retention)-Activity Relationships dla badanych grup związków. W całym cyklu publikacji wyprowadzono i poddano walidacji równania QS(R)ARs, opisujących jedenaście parametrów farmakokinetycznych: $\log BB$ [D1-D4], $\log K_{HSA}$ [D1-D3], $\log P_{w/HSA}$ [D2], $\log K_p$ [D1-D2], $Caco\text{-}2$ [D1], V_d [D2], $f_{u,brain}$ [D1-D3], $f_{u,plasma}$, $\log K_{sc}$, $\log K_{w/cell}$ i $\log P_{w/pc}$ oraz parametr toksyczności LD_{50} [D2]. Uzyskane parametry statystyczne powstałych równań pozwalają na uznanie wszystkich z wyprowadzonych zależności za bardzo dobre i wiarygodne.

Wyniki badań przesiewowych, zrealizowanych w czasie trwania projektu badawczego, prowadzą do konkluzji, że analizowane związki organiczne powinny zostać poddane dalszym badaniom klinicznym ze względu na ich wysoki potencjał zastosowania jako leki lub pestycydy. Szczególną uwagę warto zwrócić na substancje analizowane w publikacjach D1 i D3, ze względu na ich pożądane właściwości przeciwnowotworowe.

Działanie zgodnie z zasadami zielonej chemii pozwoliło na zużycie jak najmniejszej ilości materiałów i odczynników chemicznych. W celu dalszej redukcji wytwarzanych odpadów przeprowadzono również dodatkowe badania, potwierdzające możliwości kilkukrotnego wykorzystania płytek chromatograficznych [D3] oraz zastosowania tylko jednej fazy ruchomej do wyliczenia chromatograficznych parametrów lipofilowości [D1]. Takie rozwiązania pozwolą na skrócenie ilości zużytych odczynników oraz czasu analizy w przypadku intensywnie prowadzonych badań przesiewowych.

Streszczenie w języku polskim

Ważnym zadaniem współczesnej nauki jest odpowiadanie na potrzeby i problemy społeczne, takie jak głód i brak dostępu do żywności, rozprzestrzenianie się chorób, trudności z pozyskaniem leków o pożądanych właściwościach i niewykazujących szkodliwych skutków ubocznych. Częściową odpowiedź na powyższe problemy może stanowić poszukiwanie i synteza nowych substancji aktywnych biologicznie, zarówno potencjalnych leków, jak i pestycydów. Nowe środki lecznicze powinny charakteryzować się jak najlepszą skutecznością i szerokim spektrum zastosowania (przeciwwirusowym, przeciwzapalnym, przeciwnowotworowym etc.), podczas gdy środki ochrony roślin powinny zabezpieczać plony przed insektami i chorobami, nie wpływając przy tym na bioróżnorodność środowiska.

Istotnym etapem badań przesiewowych jest przewidywanie wpływu bioaktywnych substancji na ludzki organizm. Oceny potencjalnego działania takich związków na organizmy żywe dokonuje się poprzez wyznaczenie parametrów farmakokinetycznych, opisujących zdolność do przenikania przez różne bariery biologiczne: krew-mózg (log BB), woda-skóra (log K_p, log K_{sc}), czy wchłanianie zwrotne w jelitach (*Caco-2*). Ważnych informacji na temat przydatności nowo zsyntezowanych substancji dostarczają również parametry obrazujące ich zdolność do wiązania się z białkami surowicy krwi (log $P_{w/HSA}$, log $K_{a,HSA}$) i do transportu w organizmie oraz informujące o wielkości frakcji niezwiązanych ($f_{u,brain}$, $f_{u,plasma}$). Istotna jest także możliwość przewidywania ilości substancji aktywnej w organizmie na podstawie pomiaru jej chwilowego stężenia w osoczu, opisywana przez objętość dystrybucji (V_d). W przypadku środków ochrony roślin ważna jest także wiedza o ich toksyczności, wyrażanej za pomocą dawki śmiertelnej (*LD$_{50}$*) oraz o podziale substancji pomiędzy wodę a kutykulę (nabłonek) rośliny (log $P_{w/pc}$) i przenikaniu do komórek roślinnych (log $K_{w/cell}$).

Pochodne triazoli wykazują szerokie spektrum właściwości – zarówno przeciwwirusowe, przeciwgrzybiczne, przeciwbólowe, jak i przeciwnowotworowe. Rozległa możliwość zastosowania wymaga znajomości procesów metabolicznych zachodzących po ich wprowadzeniu do ludzkiego organizmu. Wiedza ta jest również ważna w przypadku pestycydów, które mogą dostać się do układu pokarmowego lub oddechowego w sposób przypadkowy. Informacja o możliwym negatywnym wpływie zażytej substancji ułatwi podjęcie dalszych odpowiednich decyzji. Analizowane w przedstawianych badaniach środki ochrony roślin zaliczają się do dwóch grup – pochodnych kwasów karbaminowego (potencjalne insektycydy) oraz fenoksyoctowego (środki chwastobójcze).

Celem projektu badawczego było zastosowanie chromatografii cieczowej i metodyki Quantitative Structure (Retention)-Activity Relationships do przewidywania właściwości farmakokinetycznych nowych substancji organicznych rozważanych jako potencjalne leki i środki ochrony roślin. W czasie badań postawiono następujące cele pośrednie: wyznaczenie chromatograficznych parametrów $\log k_w$, $\log k_m$, $\log K_{AM}$ i ich pochodnych oraz ich ocena jako deskryptorów lipofilowości substancji; przewidywanie wpływu lipofilowości właściwości fizykochemicznych badanych związków na parametry farmakokinetyczne; wyprowadzenie modeli QS(R)ARs i wskazanie najlepszych z nich do przewidywania właściwości biologicznych substancji. Praca badawcza została zrealizowana zgodnie z zasadami zielonej chemii, z ograniczeniem zużycia odczynników i materiałów chemicznych oraz wygenerowaniu jak najmniejszej ilości odpadów.

Lipofilowość jest dominującym czynnikiem spośród decydujących o aktywności biologicznej substancji. Współczynnik podziału n-oktanol/woda ($\log P_{o/w}$) jest najbardziej popularnym parametrem służącym do jej opisu, ale równocześnie bardzo trudnym do wyznaczenia metodami eksperymentalnymi. W przeprowadzonych badaniach do tego celu wykorzystano metody *in silico* (obliczeniowe) i *in vitro* (chromatograficzne). Program ACD/Percepta umożliwił wyliczenie parametrów lipofilowości $\log P_{o/w}$ na podstawie struktury substancji i zgromadzonej bazy danych. Modyfikacje fazy ruchomej i stacjonarnej w stosowanych technikach chromatograficznych pozwoliły na osiągnięcie warunków imitujących obecne w organizmach żywych. Chromatografia w odwróconym układzie faz RP LC została wykorzystana do wyprowadzenia ekstrapolowanego współczynnika retencyjnego $\log k_w$, a pomiary przeprowadzono zarówno z użyciem klasycznej kolumny RP-18e, jak i kolumny ze sztuczną membraną IAM. Badania wykorzystujące micelarną chromatografię cieczową wykonano zarówno w technice kolumnowej (kolumny RP-8e, RP-18e), jak i planarnej (płytki TLC RP-CN). Modyfikacja fazy ruchomej poprzez dodatek surfaktantów Brij 35 lub SDS umożliwiła wyznaczenie parametrów $\log k_m$, $\log K_{AM}$, $\log (k_m/K_{AM})$ oraz R_M. Uzyskane chromatograficzne parametry lipofilowości zostały porównane ze współczynnikami $\log P_{o/w}$ i na tej podstawie oceniono je jako alternatywne deskryptory lipofilowości badanych związków organicznych.

Otrzymane chromatograficzne deskryptory lipofilowości wykorzystano w metodyce Quantitative Structure (Retention)-Activity Relationships do wyprowadzenia równań łączących aktywność biologiczną substancji z jej strukturą/retencją. Dla parametrów fizykochemicznych, w których lipofilowość okazywała się decydującym czynnikiem

wpływającym na aktywność biologiczną, zaprezentowano zależności (liniowe lub paraboliczne) pomiędzy danym parametrem a współczynnikiem retencji. W pozostałych przypadkach zostały stworzone równania wielokrotnej regresji liniowej poprzez wprowadzenie do nich kolejnych zmiennych niezależnych (parametrów fizykochemicznych). Wykorzystywano parametry steryczne (masę molową, polaryzowalność, liczbę wiązań rotacyjnych) oraz elektronowe – liczbę donorów i akceptorów wiązania wodorowego, topologiczne pole powierzchni polarnej. Wszystkie wyprowadzone zależności zostały poddane walidacji oraz analizie statystycznej. Dopasowanie otrzymanych modeli do wprowadzonych danych oraz ich zdolność predykcyjną sprawdzono poprzez wyliczenie współczynników determinacji R^2, $R^2_{adj.}$ i $R^2_{pred.}$. Wykresy Williamsa zostały wykorzystane do zobrazowania dziedzin wykorzystania modelu (AD), w których powinien on umożliwiać prognozowanie z dobrym prawdopodobieństwem. Wyodrębnienie odstających danych zostało dokonane zarówno na podstawie wspomnianych wykresów, jak i w oparciu o korelacje otrzymane pomiędzy wartościami rzeczywistymi a obliczonymi na podstawie wyprowadzonych równań.

Opisane etapy badań pozwoliły na zrealizowanie wszystkich założonych celów badawczych. Wykonane pomiary chromatograficzne umożliwiły wyliczenie różnych parametrów retencyjnych, a porównanie uzyskanych danych z wartościami parametru log $P_{o/w}$ – ich ocenę jako chromatograficznych deskryptorów lipofilowości. Analiza statystyczna i walidacja wyprowadzonych równań QS(R)ARs pozwoliły na wybór najlepszych modeli. Zobrazowanie standaryzowanych współczynników za pomocą wykresów ułatwiło porównanie jakościowego i ilościowego wpływu lipofilowości i parametrów fizykochemicznych substancji na dane właściwości farmakokinetyczne. Kilkukrotne wykorzystanie tych samych płytek chromatograficznych (po ich wcześniejszym odmyciu) pozwoliło na ograniczenie zużycia materiałów i kosztów analiz.

Streszczenie w języku angielskim

Social issues and needs should be a major focus of modern science. For many years these have, unfortunately, included hunger and the lack of food availability, as well as the spread of disease and difficulty in acquiring medicines. Search for and synthesis of new bioactive compounds, especially potential pesticides and medicaments, could be a part of the solution. Newly obtained drugs should be characterized by high efficacy and a broad spectrum of applications (antiviral, anti-inflammatory, anti-cancer, etc.). Plant protection products should save crops from insects and diseases without affecting environmental biodiversity.

An important step in screening is the analysis of the side effects bioactive substances have on living organisms. The risk assessment is made by the determination of various pharmacokinetic parameters, describing their ability to penetrate biological barriers, such as: blood-brain (log BB), water-skin (log K_p, log K_{sc}), or intestinal absorption ($Caco-2$). Important information about the suitability of newly synthesized and tested substances is also provided by parameters showing their ability to bind themselves to blood proteins (log $P_{w/HSA}$, log $K_{a,HSA}$) and then move through the body, as well as descriptors providing information about the concentration of unbounded fractions ($f_{u,brain}$ and $f_{u,plasma}$). It is also important to predict the amount of active compound in the body based on the measurement of its instantaneous plasma concentration, described by the volume of distribution (V_d). With regard to potential pesticides, it is also crucial to know their toxicity, as expressed by the lethal dose (LD_{50}), their partitioning between water and plant's cuticle (log $P_{w/pc}$), and penetration into plant cells (log $K_{w/cell}$).

Triazole derivatives are characterized by their wide spectrum of properties – antiviral, antifungal, anticancer, and analgesic. Their extensive applicability demands knowledge of metabolic processes occurring after they enter the human body. The same necessity also exists in the case of pesticides which could accidentally enter the digestive or respiratory system. The awareness of a substance's potential negative impact would facilitate appropriate decisions. Tested plant protection products fall into two groups – derivatives of carbamic acids (potential insecticides) and phenoxyacetic acids (herbicides).

The main aim of the research was to apply liquid chromatography and Quantitative Structure (Retention)-Activity Relationships methods to predict the pharmacokinetic properties of new organic substances considered as potential drugs and plant protection

products. The following specific objectives were set for the project: to determine chromatographic parameters $\log k_w$, $\log k_m$, $\log K_{AM}$ and their derivatives and to assess their potential of lipophilicity descriptors; to predict physiochemical and lipophilic properties' influence on pharmacokinetic descriptors; to derive QS(R)ARs models and choose the best of these to predict biological properties of the substances. The project was carried out following green chemistry principles, reducing the use of reagents and materials, and generating as little waste as possible.

Lipophilicity is a dominant factor determining a substance's bioactivity. Partition coefficient n-octanol/water ($\log P_{o/w}$) is the most popular parameter used to describe it, but at the same time, it is very difficult to determine by experimental methods. In this research calculation (*in silico*) and chromatography (*in vitro*) methods were applied to establish compound's lipophilicity. ACD/Percepta program enabled calculating the $\log P_{o/w}$ parameter based on a substance's structure and collected dataset. Modification of both mobile and stationary phases made it possible to achieve conditions mimicking those present in living organisms. Reversed-phase liquid chromatography (RP LC) was used to compute the extrapolated retention factor $\log k_w$. The measurements were made employing both a chromatographic column RP-18e and a column with immobilized artificial membrane (IAM). The micellar liquid chromatography (MLC) method was applied with the use of both column (RP-8e, RP-18e) and planar (RP-CN) techniques. The addition of surfactant Brij 35 or SDS to effluent led to the determination of $\log k_m$, $\log K_{AM}$, $\log (k_m/K_{AM})$, and R_M parameters. All obtained chromatographic lipophilicity descriptors were compared to the $\log P_{o/w}$ parameter and the possibility of their use as alternative lipophilicity descriptors of organic compounds was evaluated.

The results obtained were then employed within the Quantitative Structure (Retention)-Activity Relationships method in order to derive equations describing the relationship between a substance's bioactivity with its structure/retention time. In cases where lipophilicity was the only factor affecting bioactivity, linear or parabolic relationships between pharmacokinetic parameters and the retention factor were presented. In all other cases, the equations of multiple linear regression were constructed by adding further independent variables (physicochemical descriptors). The steric parameters used were molecular weight, polarizability, and the number of rotation bonds; the electronic ones – the number of hydrogen bond donors and acceptors, and topological polar surface area. All obtained models were validated and statistically analyzed. The fittingness of the obtained models to the input data

and their predictive ability were checked by calculating the R^2, $R^2_{adj.}$ and $R^2_{pred.}$ determination coefficients. Williams charts were used to illustrate the areas where a model should be able to forecast with good probability. The extraction of outliers was done both on the basis of the aforementioned graphs and of the correlations obtained between actual values of the pharmacokinetic properties and those calculated from the derived equations.

The described stages of the study made it possible to achieve all the aims of the research. Chromatographic measurements enabled the calculation of various retention parameters, and the comparison of the obtained data with the values of the log $P_{o/w}$ parameter – their evaluation as chromatographic descriptors of lipophilicity. Statistical analysis and validation of the derived QSARs equations made it possible to select the best models. The standardized coefficient figures facilitated the comparison of qualitative and quantitative effects of lipophilicity and physicochemical parameters of substances on the pharmacokinetic properties. Multiple uses of the same chromatographic plates (after their previous washing) and made it possible to reduce the use of materials and organic solvents.

Literatura

Abraham, M. H., Chadha, H. S., & Mitchell, R. C. (1994). Hydrogen bonding. 33. Factors that influence the distribution of solutes between blood and brain. *Journal of Pharmaceutical Sciences, 83*(9), 1257-1268. https://doi.org/10.1002/jps.2600830915

Aggarwal, M., Fisher, P., Hüser, A., Kluxen, F. M., Parr-Dobrzanski, R., Soufi, M., Strupp, C., Wiemann, C., & Billington, R. (2015). Assessment of an extended dataset of in vitro human dermal absorption studies on pesticides to determine default values, opportunities for read-across and influence of dilution on absorption. *Regulatory Toxicology and Pharmacology, 72*(1), 58-70. https://doi.org/10.1016/j.yrtph.2015.02.017

Ajay, Bemis, G. W., & Murcko, M. A. (1999). Designing libraries with CNS activity. *Journal of Medicinal Chemistry, 42*(24), 4942-4951. https://doi.org/10.1021/jm990017w

Armstrong, D. W., & Nome, F. (1981). Partitioning behavior of solutes eluted with micellar mobile phases in liquid chromatography. *Analytical Chemistry, 53*(11), 1662-1666. https://doi.org/10.1021/ac00234a026

Arunyanart, M., & Cline Love, L. J. (1984). Model for Micellar Effects on Liquid Chromatography Capacity Factors and for Determination of Micelle-Solute Equilibrium Constants. *Analytical Chemistry, 56*(9), 1557-1561. https://doi.org/10.1021/ac00273a005

Ayouni, L., Cazorla, G., Chaillou, D., Herbreteau, B., Rudaz, S., Lantéri, P., & Carrupt, P. A. (2005). Fast determination of lipophilicity by HPLC. *Chromatographia, 62*(5–6), 251–255. https://doi.org/10.1365/s10337-005-0608-6

Azman, M., Sabri, A. H., Anjani, Q. K., Mustaffa, M. F., & Hamid, K. A. (2022). Intestinal Absorption Study: Challenges and Absorption Enhancement Strategies in Improving Oral Drug Delivery. *Pharmaceuticals, 15*(8), 975. https://doi.org/10.3390/ph15080975

Bahgat, E. A., Saleh, H., Reda, A., & Fawzy, M. G. (2022). Development and validation of eco-friendly micellar organic solvent-free HPLC method for the simultaneous determination of some antihypertensive combinations. *Microchemical Journal, 181,* 107740. https://doi.org/10.1016/j.microc.2022.107740

Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis, 6*(2), 71-79. https://doi.org/10.1016/j.jpha.2015.11.005

Barbato, F., Di Martino, G., Grumetto, L., & La Rotonda, M. I. (2004). Prediction of drug-membrane interactions by IAM-HPLC: Effects of different phospholipid stationary phases on the partition of bases. *European Journal of Pharmaceutical Sciences, 22*(4), 261-269. https://doi.org/10.1016/j.ejps.2004.03.019

Baur, P., Marzouk, H., Schönherr, J., & Grayson, B. T. (1997). Partition Coefficients of Active Ingredients between Plant Cuticle and Adjuvants as Related to Rates of Foliar Uptake. *Journal of Agricultural and Food Chemistry*, *45*(9), 3659-3665. https://doi.org/10.1021/jf970233i

Betarbet, R., Sherer, T. B., MacKenzie, G., Garcia-Osuna, M., Panov, A. V., & Greenamyre, J. T. (2000). Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nature Neuroscience*, *3*(12), 1301-1306. https://doi.org/10.1038/81834

Braumann, T. (1986). Determination of hydrophobic parameters by reversed-phase liquid chromatography: theory, experimental techniques, and application in studies on quantitative structure-activity relationships. *Journal of Chromatography A*, *373*(C), 191–225. https://doi.org/10.1016/S0021-9673(00)80213-7

Breyer, E. D., Strasters, J. K., & Khaledi, M. G. (1991). Quantitative Retention-Biological Activity Relationship Study by Micellar Liquid Chromatography. *Analytical Chemistry*, *63*(8), 828–833. https://doi.org/10.1021/ac00008a019

Camenisch, G., Alsenz, J., van de Waterbeemd, H., & Folkers, G. (1998). Estimation of permeability by passive diffusion through Caco-2 cell monolayers using the drugs' lipophilicity and molecular weight. *European Journal of Pharmaceutical Sciences*, *6*(4), 313–319. https://doi.org/10.1016/S0928-0987(97)10019-7

Chan, R., De Bruyn, T., Wright, M., & Broccatelli, F. (2018). Comparing Mechanistic and Preclinical Predictions of Volume of Distribution on a Large Set of Drugs. *Pharmaceutical Research*, *35*(4), 87-97. doi:10.1007/s11095-018-2360-2

Chen, C. P., Chen, C. C., Huang, C. W., & Chang, Y. C. (2018). Evaluating molecular properties involved in transport of small molecules in stratum corneum: A quantitative structure-activity relationship for skin permeability. *Molecules*, *23*(4), 911. https://doi.org/10.3390/molecules23040911

Chen, Y. C., Kenny, J. R., Wright, M., Hop, C. E. C. A., & Yan, Z. (2019). Improving Confidence in the Determination of Free Fraction for Highly Bound Drugs Using Bidirectional Equilibrium Dialysis. *Journal of Pharmaceutical Sciences*, *108*(3), 1296–1302. https://doi.org/10.1016/j.xphs.2018.10.011

Ciura, K., Dziomba, S., Nowakowska, J., & Markuszewski, M. J. (2017). Thin layer chromatography in drug discovery process. *Journal of Chromatography A*, *1520*, 9–22. https://doi.org/10.1016/j.chroma.2017.09.015

Clementi, M., Clementi, S., Fornaciari, M., Orlandi, F., & Romano, B. (2001). The GOLPE procedure for predicting olive crop production from climatic parameters. *Journal of Chemometrics*, *15*(4), 397–404. https://doi.org/10.1002/cem.670.abs

Collander, R., Lindholm, M., Haug, C. M., Stene, J., & Sörensen, N. A. (1951). The Partition of Organic Compounds Between Higher Alcohols and Water. *Acta Chemica Scandinavica*, *5*, 774–780. https://doi.org/10.3891/acta.chem.scand.05-0774

Czerwiński, W. (1981). *Fizjologia roślin*. Wydawnictwo Naukowe PWN.

Dahlgren, D., & Lennernäs, H. (2019). Intestinal permeability and drug absorption: predictive experimental, computational and in vivo approaches. *Pharmaceutics, 11*(8), 411. https://doi.org/10.3390/pharmaceutics11080411

Dorsey, J. G., DeEchegaray, M. T., & Landy, J. S. (1983). Efficiency Enhancement in Micellar Liquid Chromatography. *Analytical Chemistry, 55*(6), 924-928. https://doi.org/10.1021/ac00257a024

Duijsens, D., Pälchen, K., Verkempinck, S., Guevara-Zambrano, J., Hendrickx, M., Van Loey, A., & Grauwet, T. (2023). Size exclusion chromatography to evaluate in vitro proteolysis: A case study on the impact of microstructure in pulse powders. *Food Chemistry, 418*, 135709. https://doi.org/10.1016/j.foodchem.2023.135709

Eriksson, L., Jaworska, J., Worth, A. P., Cronin, M. T. D., McDowell, R. M., & Gramatica, P. (2003). Methods for reliability and uncertainty assessment and for applicability evaluations of classification- and regression-based QSARs. *Environmental Health Perspectives, 111*(10), 1361-1375. https://doi.org/10.1289/ehp.5758

Ertl, P., Rohde, B., & Selzer, P. (2000). Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *Journal of Medicinal Chemistry, 43*(20), 3714-3717. https://doi.org/10.1021/jm000942e

Fasano, M., Curry, S., Terreno, E., Galliano, M., Fanali, G., Narciso, P., Notari, S., & Ascenzi, P. (2005). The extraordinary ligand binding properties of human serum albumin. *IUBMB Life, 57*(12), 787-796. https://doi.org/10.1080/15216540500404093

Feher, M., Sourial, E., & Schmidt, J. M. (2000). A simple model for the prediction of blood-brain partitioning. *International Journal of Pharmaceutics, 201*(2), 239-247. https://doi.org/10.1016/S0378-5173(00)00422-1

Foley, J. P. (1990). Critical compilation of solute-micelle binding constants and related parameters from micellar liquid chromatographic measurements. *Analytica Chimica Acta, 231*, 237-247. https://doi.org/10.1016/S0003-2670(00)86422-3

Fridén, M., Bergström, F., Wan, H., Rehngren, M., Ahlin, G., Hammarlund-Udenaes, M., & Bredberg, U. (2010). Measurement of Unbound Drug Exposure in Brain: Modeling of pH Partitioning Explains Diverging Results Between the Brain Slice and Brain Homogenate Methods. *Drug Metabolism and Disposition, 39*(3), 353-362. https://doi.org/10.1124/dmd.110.035998

Geldenhuys, W. J., Mohammad, A. S., Adkins, C. E., & Lockman, P. R. (2015). Molecular determinants of blood-brain barrier permeation. *Therapeutic Delivery, 6*(8), 961-971. https://doi.org/10.4155/tde.15.32

Ghose, A. K., Viswanadhan, V. N., & Wendoloski, J. J. (1999). A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *Journal of Combinatorial Chemistry*, *1*(1), 55-68. https://doi.org/10.1021/cc9800071

Godard, T., & Grushka, E. (2011). The use of phospholipid modified column for the determination of lipophilic properties in high performance liquid chromatography. *Journal of Chromatography A*, *1218*(9), 1211-1218. https://doi.org/10.1016/j.chroma.2010.12.105

Goulson, D., Nicholls, E., Botías, C., & Rotheray, E. L. (2015). Bee declines driven by combined Stress from parasites, pesticides, and lack of flowers. *Science*, *347*(6229), 1255957. https://doi.org/10.1126/science.1255957

Grabowski, T. (2015). *Farmakokinetyka i biofarmacja*. Tomasz Grabowski

Gramatica, P. (2012). On the development and validation of QSAR models. *Computational Toxicology*, 499-526. https://doi.org/10.1007/978-1-62703-059-5_21

Grassy, G., & Chavanieu, A. (2011). Molecular Lipophilicity: A Predominant Descriptor for QSAR. In *Chemogenomics and Chemical Genetics*, 153-170. https://doi.org/10.1007/978-3-642-19615-7_12

Hansch, C. (1969). A Quantitative Approach to Biochemical Structure-Activity Relationships. *Accounts of Chemical Research*, *2*(8), 232-239. https://doi.org/10.1021/ar50020a002

Hansch, C. (1993). Quantitative Structure-Activity Relationships and the Unnamed Science. *Accounts of Chemical Research*, *26*(4), 147-153. https://doi.org/10.1021/ar0028a003

Héberger, K. (2007). Quantitative structure-(chromatographic) retention relationships. In *Journal of Chromatography A*, *1158*(1-2), 273-305. https://doi.org/10.1016/j.chroma.2007.03.108

How to Interpret Adjusted R-Squared and Predicted R-Squared in Regression Analysis. (n.d.). Statistics by Jim. https://statisticsbyjim.com/regression/interpret-adjusted-r-squared-predicted-r-squared-regression/

Iyer, M., Mishra, R., Han, Y., & Hopfinger, A. J. (2002). Predicting blood-brain barrier partitioning of organic molecules using membrane-interaction QSAR analysis. *Pharmaceutical Research*, *19*(11), 1611-1621. https://doi.org/10.1023/A:1020792909928

Janicka, M., Sztanke, M., & Sztanke, K. (2013). Reversed-phase liquid chromatography with octadecylsilyl, immobilized artificial membrane and cholesterol columns in correlation studies with in silico biological descriptors of newly synthesized antiproliferative and analgesic active compounds. *Journal of Chromatography A*, *1318*, 92-101. https://doi.org/10.1016/j.chroma.2013.09.060

Joko, T., Sulistiyani, Setiani, O., Rahardjo, M., & Arumdani, I. S. (2023). Life Cycle Analysis on Pesticide Exposure and Residues in the Environment of Brebes County Shallot Farms and Farmers. *Journal of Ecological Engineering*, *24*(3), 76-89. https://doi.org/10.12911/22998993/157424

Kaliszan, R. (1987). *Quantitative Structure-Chromatographic Retention Relationships*. J. Wiley & Sons.

Kaliszan, R., Van Straten, M. A., Markuszewski, M., Cramers, C. A., & Claessens, H. A. (1999). Molecular mechanism of retention in reversed-phase high-performance liquid chromatography and classification of modern stationary phases by using quantitative structure-retention relationships. *Journal of Chromatography A, 855*(2), 455-486. https://doi.org/10.1016/S0021-9673(99)00742-6

Kalyankar, T. M., Kulkarni, P. D., Wadher, S. J., & Pekamwar, S. S. (2014). Applications of micellar liquid chromatography in bioanalysis: A review. *Journal of Applied Pharmaceutical Science, 4*(1), 128-134. https://doi.org/10.7324/JAPS.2014.40122

Karlsson, J. O. G. (1996). Preclinical safety assessment of contrast media: Predictive value. *European Radiology, 6*(S2). https://doi.org/10.1007/bf02342564

Kelder, J., Grootenhuis, P. D. J., Bayada, D. M., Delbressine, L. P. C., & Ploemen, J. P. (1999). Polar molecular surface as a dominating determinant for oral absorption and brain penetration of drugs. *Pharmaceutical Research, 16*(10), 1514-1519. https://doi.org/10.1023/A:1015040217741

Kerns, E. H., & Di, L. (2003). Pharmaceutical profiling in drug discovery. *Drug Discovery Today, 8*(7), 316-323. https://doi.org/10.1016/S1359-6446(03)02649-7

Komsta, Ł., Waksmundzka-Hajnos, M., Sherma, J. (Red.). (2013). *Thin Layer Chromatography in Drug Analysis*. CRC Press.

Kord, A. S., & Khaledi, M. G. (1993). Selectivity of organic solvents in micellar liquid chromatography of amino acids and peptides. *Journal of Chromatography A, 631*(1–2), 125-132. https://doi.org/10.1016/0021-9673(93)80512-7

Kraft, T. E., Richter, W. F., Emrich, T., Knaupp, A., Schuster, M., Wolfert, A., & Kettenberger, H. (2020). Heparin chromatography as an in vitro predictor for antibody clearance rate through pinocytosis. *MAbs, 12*(1), 1683432. https://doi.org/10.1080/19420862.2019.1683432

Lentz, K., Raybon, J., & Sinz, M. W. (2013). Drug Metabolism and Pharmacokinetics in Drug Discovery. *Drug Discovery: Practices, Processes, and Perspectives*, 99-139. https://doi.org/10.1002/9781118354483.ch4

Lipinski, C. A., Lombardo, F., Dominy, B. W., & Feeney, P. J. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Advanced Drug Delivery Reviews, 46*, 3-26. https://doi.org/10.1016/s0169-409x(00)00129-0

Liu, R., Sun, H., & So, S. S. (2001). Development of Quantitative Structure - Property Relationship Models for Early ADME Evaluation in Drug Discovery. 2. Blood-Brain Barrier Penetration. *Journal of Chemical Information and Computer Sciences, 41*(6), 1623-1632. https://doi.org/10.1021/ci010290i

López Grío, S., Baeza Baeza, J. J., & García Alvarez-Coque, M. C. (1999). Modelling of the elution behaviour in hybrid micellar eluents with different organic modifiers. *Analytica Chimica Acta, 381*(2–3), 276-285. https://doi.org/10.1016/S0003-2670(98)00704-1

Matsuzaki, R., Gunnigle, E., Geissen, V., Clarke, G., Nagpal, J., & Cryan, J. F. (2023). Pesticide exposure and the microbiota-gut-brain axis. *ISME Journal.* https://doi.org/10.1038/s41396-023-01450-9

Mouritsen, O. G., & Jørgensen, K. (1998). A new look at lipid-membrane structure in relation to drug research. *Pharmaceutical Research, 15*(10), 1507-1519. https://doi.org/10.1023/A:1011986613392

Nasal, A., Siluk, D., & Kaliszan, R. (2012). Chromatographic Retention Parameters in Medicinal Chemistry and Molecular Pharmacology. *Current Medicinal Chemistry, 10*(5), 381-426. https://doi.org/10.2174/0929867033368268

OECD Council. (1989). *OECD GUIDELINE FOR TESTING OF CHEMICALS Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method.* https://www.oecd.org/chemicalsafety/risk-assessment/1948177.pdf

OECD Council. (2007). *GUIDANCE DOCUMENT ON THE VALIDATION OF (QUANTITATIVE) STRUCTURE-ACTIVITY RELATIONSHIP [(Q)SAR] MODELS.*

Oliveira, T. M. B. F., Ribeiro, F. W. P., Sousa, C. P., Salazar-Banda, G. R., de Lima-Neto, P., Correia, A. N., & Morais, S. (2020). Current overview and perspectives on carbon-based (bio)sensors for carbamate pesticides electroanalysis. *TrAC - Trends in Analytical Chemistry, 124*, 115779. https://doi.org/10.1016/j.trac.2019.115779

O'Shea, J. P., Augustijns, P., Brandl, M., Brayden, D. J., Brouwers, J., Griffin, B. T., Holm, R., Jacobsen, A. C., Lennernäs, H., Vinarov, Z., & O'Driscoll, C. M. (2022). Best practices in current models mimicking drug permeability in the gastrointestinal tract - An UNGAP review. *European Journal of Pharmaceutical Sciences, 170*, 106098. https://doi.org/10.1016/j.ejps.2021.106098

Peris-García, E., Ruiz-Angel, M. J., Carda-Broch, S., & García-Alvarez-Coque, M. C. (2017). Analysis of basic drugs by liquid chromatography with environmentally friendly mobile phases in pharmaceutical formulations. *Microchemical Journal, 134*, 202–210. https://doi.org/10.1016/j.microc.2017.06.009

Platts, J. A., Abraham, M. H., Hersey, A., & Butina, D. (2000). Estimation of molecular linear free energy relationship descriptors. 4. Correlation and prediction of cell permeation. *Pharmaceutical Research, 17*(8), 1013-1018. https://doi.org/10.1023/A:1007543708522

Pourbasheer, E., Riahi, S., Ganjali, M. R., & Norouzi, P. (2010). Quantitative structureactivity relationship (QSAR) study of interleukin-1 receptor associated kinase 4 (IRAK-4) inhibitor activity by the genetic algorithm and multiple linear regression (GA-MLR) method. *Journal of Enzyme Inhibition and Medicinal Chemistry, 25*(6), 848-853. https://doi.org/10.3109/14756361003757893

Prausnitz, M. R., & Langer, R. (2008). Transdermal drug delivery. *Nature Biotechnology*, 26(11), 1261-1268. https://doi.org/10.1038/nbt.1504

Quiñones-Torrelo, C., Martin-Biosca, Y., Martinez-pla, J., Sagrado, S., Villanueva-Camanas, R., & Medina-Hernandez, M. (2005). QRAR Models for Central Nervous System Drugs using Biopartitioning Micellar Chromatography. *Mini-Reviews in Medicinal Chemistry*, 2(2), 145–161. https://doi.org/10.2174/1389557024605519

Quiñones-Torrelo, C., Sagrado-Vives, S., Villanueva-Camañas, R. M., & Medina-Hernández, M. J. (2001). An LD50 model for predicting psychotropic drug toxicity using biopartitioning micellar chromatography. *Biomedical Chromatography*, 15(1), 31-40. https://doi.org/10.1002/bmc.24.abs

Rambla-Alegre, M. (2012). Basic Principles of MLC. *Chromatography Research International*, 2012, 1-6. https://doi.org/10.1155/2012/898520

Rein, M. J., Renouf, M., Cruz-Hernandez, C., Actis-Goretta, L., Thakkar, S. K., & da Silva Pinto, M. (2013). Bioavailability of bioactive food compounds: A challenging journey to bioefficacy. *British Journal of Clinical Pharmacology*, 75(3), 588-602. https://doi.org/10.1111/j.1365-2125.2012.04425.x

Roberts, S. A. (2003). Drug metabolism and pharmacokinetics in drug discovery. *Current Opinion in Drug Discovery and Development*, 6(1), 66-80. https://pubmed.ncbi.nlm.nih.gov/12613278/

Roy, K., Kar, S., & Ambure, P. (2015). On a simple approach for determining applicability domain of QSAR models. *Chemometrics and Intelligent Laboratory Systems*, 145, 22–29. https://doi.org/10.1016/j.chemolab.2015.04.013

Ruiz-Ángel, M. J., Garcia-Álvarez-Coque, M. C., & Berthod, A. (2009). New insights and recent developments in micellar liquid chromatography. *Separation and Purification Reviews*, 38(1), 45-96. https://doi.org/10.1080/15422110802178876

Ruiz-Garcia, A., Bermejo, M., Moss, A., & Casabo, V. G. (2008). Pharmacokinetics in drug discovery. *Journal of Pharmaceutical Sciences*, 97(2), 654-690. https://doi.org/10.1002/jps.21009

Rutkowska, E., Pajak, K., & Jóźwiak, K. (2013). Lipophilicity – methods of determination and its role in medicinal chemistry. *Acta Poloniae Pharmaceutica*, 70(1), 3-18. https://pubmed.ncbi.nlm.nih.gov/23610954/

Sagandykova, G. N., Pomastowski, P. P., Kaliszan, R., & Buszewski, B. (2018). Modern analytical methods for consideration of natural biological activity. *TrAC - Trends in Analytical Chemistry*, 109, 198-213. https://doi.org/10.1016/j.trac.2018.10.012

Sahigara, F., Mansouri, K., Ballabio, D., Mauri, A., Consonni, V., & Todeschini, R. (2012). Comparison of different approaches to define the applicability domain of QSAR models. *Molecules*, 17(5), 4791–4810. https://doi.org/10.3390/molecules17054791

Seddon, A. M., Casey, D., Law, R. V., Gee, A., Templer, R. H., & Ces, O. (2009). Drug interactions with lipid membranes. *Chemical Society Reviews*, *38*(9), 2509. https://doi.org/10.1039/b813853m

Soares, J. X., Santos, Á., Fernandes, C., & Pinto, M. M. M. (2022). Liquid Chromatography on the Different Methods for the Determination of Lipophilicity: An Essential Analytical Tool in Medicinal Chemistry. *Chemosensors*, *10*(8), 340. https://doi.org/10.3390/chemosensors10080340

Soukup, J., & Jandera, P. (2012). Hydrosilated silica-based columns: The effects of mobile phase and temperature on dual hydrophilic-reversed-phase separation mechanism of phenolic acids. *Journal of Chromatography A*, *1228*, 125-134. https://doi.org/10.1016/j.chroma.2011.06.077

Struger, J., Grabuski, J., Cagampan, S., Sverko, E., & Marvin, C. (2016). Occurrence and Distribution of Carbamate Pesticides and Metalaxyl in Southern Ontario Surface Waters 2007-2010. *Bulletin of Environmental Contamination and Toxicology*, *96*(4), 423-431. https://doi.org/10.1007/s00128-015-1719-x

Sztanke, K., Tuzimski, T., Sztanke, M., Rzymowska, J., & Pasternak, K. (2011). Synthesis, structure elucidation, determination of the lipophilicity and identification of antitumour activities in vitro of novel 3-(2-furanyl)-8-aryl- 7,8-dihydroimidazo[2,1-c][1,2,4]triazin-4(6H)-ones with a low cytotoxicity towards normal human skin fibroblast cells. *Bioorganic and Medicinal Chemistry*, *19*(17), 5103-5116. https://doi.org/10.1016/j.bmc.2011.07.027

Sztanke, M., Rzymowska, J., Janicka, M., & Sztanke, K. (2019). Synthesis, structure confirmation, identification of in vitro antiproliferative activities and correlation of determined lipophilicity parameters with in silico bioactivity descriptors of two novel classes of fused azaisocytosine-like congeners. *Arabian Journal of Chemistry*, *12*(8), 5302-5324. https://doi.org/10.1016/j.arabjc.2016.12.024

Sztanke, M., Sztanke, K., Rajtar, B., Świątek, Ł., Boguszewska, A., & Polz-Dacewicz, M. (2019). The influence of some promising fused azaisocytosine-containing congeners on zebrafish (Danio rerio) embryos/larvae and their antihaemolytic, antitumour and antiviral activities. *European Journal of Pharmaceutical Sciences*, *132*, 34-43. https://doi.org/10.1016/j.ejps.2019.02.033

Tesseromatis, C., & Alevizou, A. (2008). The role of the protein-binding on the mode of drug action as well the interactions with other drugs. *European Journal of Drug Metabolism and Pharmacokinetics*, *33*(4), 225-230. https://doi.org/10.1007/BF03190876

Top 20 Current Global Issues We Must Address. (n.d.). Human Rights Careers. https://www.humanrightscareers.com/issues/current-global-issues/

Toutain, P. L., & Bousquet-Mélou, A. (2004). Volumes of distribution. *Journal of Veterinary Pharmacology and Therapeutics, 27*(6), 441–453. doi:10.1111/j.1365-2885.2004.00602.x

Tsopelas, F., Danias, P., Pappa, A., & Tsantili-Kakoulidou, A. (2020). Biopartitioning micellar chromatography under different conditions: Insight into the retention mechanism and the potential to model biological processes. *Journal of Chromatography A, 1621*, 461027. https://doi.org/10.1016/j.chroma.2020.461027

Valkó, K. L. (2016). Lipophilicity and biomimetic properties measured by HPLC to support drug discovery. *Journal of Pharmaceutical and Biomedical Analysis, 130*, 35–54. https://doi.org/10.1016/j.jpba.2016.04.009

Van De Waterbeemd, H., Camenisch, G., Folkers, G., Chretien, J. R., & Raevsky, O. A. (1998). Estimation of blood-brain barrier crossing of drugs using molecular size and shape, and H-bonding descriptors. *Journal of Drug Targeting, 6*(2), 151-165. https://doi.org/10.3109/10611869808997889

Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W., & Kopple, K. D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. *Journal of Medicinal Chemistry, 45*(12), 2615-2623. https://doi.org/10.1021/jm020017n

Vuignier, K., Schappler, J., Veuthey, J. L., Carrupt, P. A., & Martel, S. (2010). Drug-protein binding: A critical review of analytical tools. *Analytical and Bioanalytical Chemistry, 398*(1), 53-66. https://doi.org/10.1007/s00216-010-3737-1

Wolak, D. J., & Thorne, R. G. (2013). Diffusion of macromolecules in the brain: Implications for drug delivery. *Molecular Pharmaceutics, 10*(5), 1492-1504. https://doi.org/10.1021/mp300495e

Yamasaki, K., Chuang, V. T. G., Maruyama, T., & Otagiri, M. (2013). Albumin-drug interaction and its clinical implication. *Biochimica et Biophysica Acta - General Subjects, 1830*(12), 5435-5443. https://doi.org/10.1016/j.bbagen.2013.05.005

Yang, F., Zhang, Y., & Liang, H. (2014). Interactive association of drugs binding to human serum albumin. *International Journal of Molecular Sciences, 15*(3), 3580-3595. https://doi.org/10.3390/ijms15033580

Pozostałe osiągnięcia

1. Konferencje międzynarodowe
   - Śliwińska, A., * & Janicka, M. Predicting lipophilicity and bioactivity of potential herbicides using RP LC and MLC techniques.
     Agriculture & Food, Burgas, 16 – 19.08.2021
   - Janicka, M., & Śliwińska, A.* MLC technique QRARs/QSARs models to predict different properties of potential pesticides.
     33rd International Symposium on Chromatography, Budapeszt, 18 – 22.09.2022
   - Janicka, M., Śliwińska, A.,* Sztanke, M., & Sztanke, K. Predicting pharmacokinetic properties of potential anticancer agents *via* their chromatographic behaviour on different reversed-phase materials
     33rd International Symposium on Chromatography, Budapeszt, 18 – 22.09.2022

2. Konferencje krajowe
   - Śliwińska, A.,* Janicka, M., Sztanke, M., & Sztanke, K. Predicting pharmacokinetic properties of potential anticancer agents *via* their chromatographic behaviour on different reversed-phase materials
     12. Polska Konferencja Chromatograficzna, Opole, 23 – 27.06.2021
   - Janicka, M., Śliwińska, A., & Malinowska, I.* Zastosowanie metodyki QR(S)ARs do przewidywania właściwości farmakokinetycznych potencjalnych pestycydów
     64. Zjazd Naukowy PTCHem, Lublin, 11 – 16.09.2022
   - Janicka, M., Śliwińska, A., Sztanke, M., Sztanke, K., & Stępnik, K.* Zastosowanie micelarnej TLC do przewidywania właściwości farmakokinetycznych potencjalnych leków przeciwnowotworowych
     64. Zjazd Naukowy PTCHem, Lublin, 11 – 16.09.2022

3. Szkolenia
   - Introduction to Molecular Modeling in Drug Discovery
     Organizator: Schrödinger; 04.2021
   - Statistics for analytical scientists
     Organizator: UK National Measurement Laboratory, 04.2022

* osoba prezentująca poster

Załączniki publikacji

D1. Janicka, M., **Mycka, A.**, Sztanke, M., & Sztanke, K. (2021). Predicting pharmacokinetic properties of potential anticancer agents via their chromatographic behavior on different reversed phase materials. *International Journal of Molecular Sciences*, *22*(8), 4257-4270. https://doi.org/10.3390/ijms22084257
IF = 6,208; PK_{MEiN} = 140

D2. Janicka, M., & **Śliwińska, A.** (2022). Quantitative retention (structure)–activity relationships in predicting the pharmaceutical and toxic properties of potential pesticides. *Molecules*, *27*(11), 3599-3618. https://doi.org/10.3390/molecules27113599
IF = 4,927; PK$_{MEiN}$ = 140

D3. Janicka, M., **Śliwińska, A.,** Sztanke, M., & Sztanke, K. (2022a). Predicting pharmacokinetic properties of potential anti-cancer agents using micellar thin-layer chromatography. *JPC – Journal of Planar Chromatography – Modern TLC, 35,* 265-272. https://doi.org/10.1007/s00764-022-00174-4

IF = 1,088; PK$_{MEiN}$ = 20

D4. Janicka, M., **Śliwińska, A.**, Sztanke, M., & Sztanke, K. (2022b). Combined micellar liquid chromatography technique and QSARs modeling in predicting the blood–brain barrier permeation of heterocyclic drug-like compounds. *International Journal of Molecular Sciences*, *23*(24), 15887-15914. https://doi.org/10.3390/ijms232415887

IF = 6,208; PK_{MEiN} = 140

Article

Predicting Pharmacokinetic Properties of Potential Anticancer Agents via Their Chromatographic Behavior on Different Reversed Phase Materials

Małgorzata Janicka [1], Anna Mycka [2], Małgorzata Sztanke [3,*] and Krzysztof Sztanke [4,*]

1 Department of Physical Chemistry, Faculty of Chemistry, Institute of Chemical Science, Maria Curie-Skłodowska University, Maria Curie-Skłodowska Sq. 3, 20-031 Lublin, Poland; malgorzata.janicka@poczta.umcs.lublin.pl

2 Doctoral School of Quantitative and Natural Sciences, Maria Curie-Skłodowska University, Maria Curie-Skłodowska Sq. 3, 20-031 Lublin, Poland; a.e.mycka@gmail.com

3 Chair and Department of Medical Chemistry, Medical University of Lublin, 4A Chodźki Street, 20-093 Lublin, Poland

4 Laboratory of Bioorganic Synthesis and Analysis, Chair and Department of Medical Chemistry, Medical University of Lublin, 4A Chodźki Street, 20-093 Lublin, Poland

* Correspondence: malgorzata.sztanke@umlub.pl (M.S.); krzysztof.sztanke@umlub.pl (K.S.); Tel.: +48-814486195 (M.S. & K.S.)

Abstract: The Quantitative Structure-Activity Relationship (QSAR) methodology was used to predict biological properties, i.e., the blood–brain distribution (log BB), fraction unbounded in the brain ($f_{u,brain}$), water-skin permeation (log K_p), binding to human plasma proteins (log $K_{a,HSA}$), and intestinal permeability (Caco-2), for three classes of fused azaisocytosine-containing congeners that were considered and tested as promising drug candidates. The compounds were characterized by lipophilic, structural, and electronic descriptors, i.e., chromatographic retention, topological polar surface area, polarizability, and molecular weight. Different reversed-phase liquid chromatography techniques were used to determine the chromatographic lipophilicity of the compounds that were tested, i.e., micellar liquid chromatography (MLC) with the ODS-2 column and polyoxyethylene lauryl ether (Brij 35) as the effluent component, an immobilized artificial membrane (IAM) chromatography with phosphatidylcholine column (IAM.PC.DD2) and chromatography with end-capped octadecylsilyl (ODS) column using aqueous solutions of acetonitrile as the mobile phases. Using multiple linear regression, we derived the statistically significant quantitative structure-activity relationships. All these QSAR equations were validated and were found to be very good. The investigations highlight the significance and possibilities of liquid chromatographic techniques with three different reversed-phase materials and QSARs methods in predicting the pharmacokinetic properties of our important organic compounds and reducing unethical animal testing.

Keywords: micellar chromatography; immobilized artificial membrane chromatography; RP-18e; QSARs; log K_p; log $K_{a,HSA}$; log BB; Caco-2; $f_{u,brain}$; fused azaisocytosine-containing congeners

1. Introduction

The use of various chromatographic techniques in supporting the drug discovery process and in physicochemical research has become quite extensive. The search for new biologically active substances, considered as potential drugs or plant protection products, is an important task in modern science. The goals are to improve people's quality of life and their life expectancy and to increase agricultural productivity while ensuring diversity and protecting the environment. One way to achieve the above goal is to synthesize new compounds that have the desired properties. Since the 19th century, it has been known that the properties of chemical substances are closely related to their molecular structures. The intensive development of the Quantitative Structure-Activity Relationships

(QSARs) method began in the 1960s and continues today [1–3]. In this method, searches are conducted to identify the multidimensional relationships that exist between the biological properties and structural parameters for a group of congeneric compounds. The derived mathematical model can be extended to new compounds with similar structures and used to predict their biological properties. In this way, it is possible to design new molecules that have the desired properties. The model becomes the basis for making decisions concerning the synthesis of new compounds, which allows researchers to limit the time and cost associated with their research. In addition, the interpretation of a mathematical model can lead to an overall model of a given biological property, which provides information that can be used to obtain the optimal design of desired chemical substances.

The relationship between solute activity and the parameters describing its molecular properties can be reported as a multiple linear regression (MLR) [2–4]:

$$Activity = aA + bB + cC + \ldots + \text{const} = f(\text{lipophilic, electronic, steric properties}) \quad (1)$$

where a, b, c, and so on are the correlation factors. The molecular descriptors $(A, B, C, \ldots)$ relating to the lipophilic, electronic, and steric properties of the molecule can be determined experimentally or evaluated in silico. Currently, there are many software products on the market that allow such calculations, e.g., HyperChem, ACD/ChemSketch, ACD/LADME, and SciLogP.

The lipophilicity of a bioactive compound is usually expressed by the logarithm of the partition coefficient in an n-octanol/water system and is either measured experimentally by the "shake-flask" method (log $P_{o/w}$) or evaluated in silico using different algorithms (fragment, atomic, molecular, or combined atomic-fragment) from molecular structures. Retention parameters, especially log k_w values, measured by a column or by planar Reversed-Phase Liquid Chromatography (RPLC), are the most popular, and they are accepted as chromatographic lipophilicity descriptors by the Organization of Economic Co-operation and Development. In addition, liquid chromatography techniques are very popular as indirect in vitro methods for the determination of lipophilicity [5–7]. The chromatographic methods used to assess lipophilicity have significant advantages, e.g., simplicity and reduced experimental time, good reproducibility, process automation, no need for quantitative analysis, and small amounts of samples required. The chromatographic methods also provide independent measurements of the low solubility of the compound and measurements of impurities or degradation products. However, some limitations of the RPLC method have also been noted. The most important of these limitations are: (1) Insufficient modeling of the n-octanol-water system for structurally diverse compounds, (2) the effect of the pore size and possible interactions with the residual silanol groups on the surface that do not occur in the n-octanol-water partitioning system, (3) time-consuming isocratic measurements, and (4) a limited working range of pH. Some solutions have been developed in the last few decades to overcome these limitations. First, it is worth mentioning the novel types of columns that were designed to mimic the n-octanol-water system (e.g., polymeric reversed-phase columns (PLRPs) or polymer-based columns) or to mimic biological partitioning, e.g., immobilized artificial membranes (IAMs) or columns with immobilized cholesterol, human or rat serum albumins, glycoproteins, and others [8–10]. Modifying the mobile phase is another solution. Such possibilities offer Micellar Liquid Chromatography (MLC) using surfactants as components of the mobile phase [5,11–13] and ionic liquids as effluents [14]. A specific type of micellar chromatography is Biopartitioning Micellar Chromatography (BMC), in which Brij 35 and a low concentration of alcohol, if necessary, are added to the mobile phase. The hydrophilic/hydrophobic nature of the surfactant in the modified stationary phase structurally resembles the ordered array of the hydrocarbon chains in the biomembranes. In addition, the surfactant and alcohol that are present in the mobile phase provide the opportunity for hydrogen bonds to form. This technique is usually referred to as Biopartitioning Micellar Chromatography [15,16] due to its similarity with biological barriers and extracellular fluids. An important advantage of
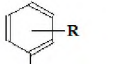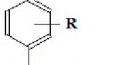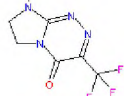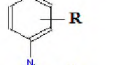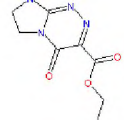
MLC is that it meets the recommendations of green chemistry by limiting the consumption of organic solvents [17].

The most important biological properties of substances considered as potential drugs are their ability to bind blood proteins (albumin and alpha-1-acid glycoprotein); penetrate biological barriers, e.g., the blood–brain barrier (BBB); permeate the skin; and perform intestinal absorption. When entering into plasma, most compounds bind rapidly to the constituents of blood, but the concentration of a free drug is responsible for the pharmacological activity, safety, and distribution of the tissue. So, the extent of protein binding in plasma affects the pharmacokinetic characteristics of a compound, i.e., its clearance, volume of distribution, half-life, drug-drug interaction, and pharmacological efficacy. Agents intended to interact with the central nervous system must be able to cross the BBB, and satisfactory transport through the blood–brain barrier is an essential prerequisite for a potential drug to affect the central nervous system. However, in order to avoid side effects, the agents that act peripherally should not cross the BBB. In both cases, the permeability of the BBB must be known and should be evaluated at the earliest possible stage of testing. Intestinal absorption is particularly important for oral medications that are transported into the blood via the intestinal tract [11,18]. Conventionally, biomimetic descriptors require animal testing (e.g., rats, dogs, monkeys, or humans). In vivo tests are extremely unethical and inhumane. They also require significant financial outlays and time that are inconsistent with the results that are achieved. Over the past few decades, along with the rapid development of new computational discoveries, combinatorial chemistry, and high-throughput biological research, it has become possible to accelerate the selection of "ideal" drug candidates for further development. If the structure of a compound is known, then it is possible to predict its lipophilic, biological, and physicochemical properties. However, in silico methods do not provide reliable results for substances with more complex structures. Compared with conventional methods, chromatography using biomimetic systems, recognized as an in vitro technique, is becoming increasingly popular.

The compounds that were tested 1–19 belong to three anticancer active classes of structurally related small molecules (Table 1) that share the same privileged heterocyclic scaffold [19–21]. Moreover, two classes of compounds possess isosteric groups such as the isopropyl in 1–6 and trifluoromethyl in 7–14. Two novel sets of fused azaisocytosine-containing congeners 1–6 and 7–14 are antimetabolites that possess the elucidated mechanism of their antiproliferative action (by caspase activation). They were synthesized in our laboratory and patented. These azaisocytosine-containing congeners were described in our earlier paper in which their medical anticancer utility was also mentioned [19]. Most of the title molecules exhibited more potent cytotoxicity in human cancer cells than a clinically approved anticancer agent, pemetrexed, and also revealed similar or more protective effects than that of ascorbic acid and Trolox in an ex vivo model of rat erythrocytes exposed to oxidants. The compounds 8, 10, 11, 17, and 19 exhibited the clearly higher antiproliferative effects in cancer cells than in normal cells [19–21]. In addition, the compounds 7, 8, and 10–12 were shown to be safer for the early life stages of *Danio rerio* than pemetrexed [22]. Due to the important pharmacological activity, all these organic substances require more thorough and extensive research on modeling their pharmacokinetic properties.

Table 1. The compounds tested and their structure, molecular weight (*MW*), topological polar surface area (*TPSA*), polarizability (α), pharmacokinetic parameters (log K_{p}, log $K_{\mathrm{a,HSA}}$, log *BB*, Caco-2, $f_{\mathrm{u.brain}}$), and lipophilicity (log *P*).

No	R	Structure	*MW* [g/mol]	*TPSA* [A²]	α [A³]	log K_{p}	log $K_{\mathrm{a,HSA}}$	log *BB*	Caco-2 E06 [cm/s]	$f_{\mathrm{u,brain}}$	log *P*
1	H		256.30	48.27	29.30	−6.061	4.80	0.230	187	0.52	2.089
2	4-CH$_3$		270.33	48.27	31.06	−5.711	4.86	0.423	217	0.32	2.701
3	2-Cl		290.75	48.27	31.13	−5.800	5.09	0.339	215	0.33	2.608
4	3-Cl		290.75	48.27	31.13	−5.645	5.07	0.384	221	0.28	2.757
5	4-Cl		290.75	48.27	31.13	−5.817	5.16	0.328	208	0.38	2.559
6	3,4-Cl$_2$		325.19	48.27	32.95	−5.370	5.44	0.473	230	0.21	3.250
7	H		282.22	48.27	25.85	−6.021	4.96	0.102	196	0.44	1.661
8	2-CH$_3$		296.25	48.27	27.61	−5.672	5.01	0.290	220	0.27	2.273
9	4-CH$_3$		296.25	48.27	27.61	−5.672	5.01	0.290	220	0.27	2.273
10	2-OCH$_3$		312.25	57.50	28.16	−6.245	5.03	0.063	195	0.43	1.647
11	2-Cl		316.67	48.27	27.68	−5.762	5.29	0.211	220	0.27	2.151
12	3-Cl		316.67	48.27	27.68	−5.603	5.31	0.264	221	0.26	2.345
13	4-Cl		316.67	48.27	27.68	−5.778	5.33	0.194	218	0.29	2.131
14	3,4-Cl$_2$		351.11	48.27	29.50	−5.332	5.64	0.345	235	0.14	2.828
15	H		286.29	74.57	30.27	−6.989	5.04	−0.243	134	0.73	0.931
16	4-CH$_3$		300.31	74,57	32.02	−6.640	5.07	−0.051	169	0.59	1.548
17	3-Cl		320.73	74,57	32.09	−6.575	5.25	−0.090	179	0.55	1.605
18	4-Cl		320.73	74.57	32.09	−6.746	5.35	−0.151	170	0.59	1.401
19	3,4-Cl$_2$		355.18	74,57	33.91	−6.300	5.60	0.000	202	0.42	2.132

In our present research, we used the following protocol: (**1**) The in vitro determination of chromatography-based lipophilicity parameters of the tested compounds using reversed-phase materials capable of imitating pharmacokinetic and partitioning processes in biological systems and an end-capped ODS column; (**2**) the in silico calculation of structural and electronic descriptors (topological polar surface area, polarizability, and molecular weight); (**3**) the in silico calculation of partition coefficients (log *P*) and pharmacokinetic properties (e.g., log *BB*, $f_{u,brain}$, log K_p, log $K_{a,HSA}$, and Caco-2) affecting drug-like properties of the tested compounds from molecular structures using an ACD/Percepta software; (**4**) the establishment and validation of new QSAR models, which make it possible to predict the pharmacokinetic properties (such as log *BB*, $f_{u,brain}$, log K_p, log $K_{a,HSA}$, and Caco-2) of the title compounds on the basis of their experimental lipophilicity parameters and structural and electronic descriptors; and (**5**) the visualization of correlations between the dependent solute properties obtained from newly constructed QSAR models and those established in silico.

2. Results

2.1. Chromatographic Data

There are several theories that describe the effect of the concentration of the surfactant in the effluent on the retention of the solute in MLC [23]. The following Foley's equation is best known in lipophilicity studies [24]:

$$\frac{1}{k} = \frac{1}{k_m} + \frac{K_{AM}}{k_m}[M] \qquad (2)$$

where [*M*] is the total concentration of the surfactant in the mobile phase minus *CMC*; K_{AM} is the constant that describes solute-micelle binding; and k_m is the solute retention parameter at the micellar concentration of zero, i.e., when the concentration of the surfactant monomer is equal to *CMC*. The K_{AM} and k_m parameters can be evaluated from the slope and intercept of the experimental $1/k$ vs. [*M*] relationships.

Equation (2) describes a linear dependence between decreasing retention and increasing micelle concentration. This equation is valid for aqueous solutions of surfactant or mobile phases with the same concentrations of the organic modifier. The micellar retention parameter, log k_m, is considered analogous to the log k_w parameter evaluated in RPLC. According to the information presented above, this parameter is considered to be a lipophilicity descriptor, and Equation (2) is a simple way to indirectly determine the lipophilic properties of compounds. It is postulated that retention in micellar chromatography depends on hydrophobic (lipophilic), electronic, and steric features of the compounds in a similar way as has been noted concerning pharmacokinetic phenomena. The additional similarity results from the fact that the phospholipids, cholesterol, fatty acids, and triglycerides included in the extracellular and intracellular fluids also form micelles with proteins.

In our investigations, the micellar retention factors were used to calculate the log k_m parameters using Equation (2) (Table 2). For all of the compounds 1-19, the relationships of $1/k$ vs. [*M*] were obtained with very good linearity, confirming that Foley's equation correctly describes the effect of the concentration of the surfactant in the effluent on the retention of the solute. In our studies, the micellar log k_m parameters for three pharmacologically active sets of compounds 1-6, 7-14, and 15-19 and the log *k* factors for solutes 15-19 obtained on IAM and end-capped ODS stationary phases were determined experimentally. All these retention factors, together with the log k_w values for compounds 1-14 obtained in our earlier investigations on IAM and end-capped ODS stationary phases [19], were used as lipophilicity descriptors in the QSARs methodology to predict the pharmacokinetic descriptors of the compounds that were tested.

Table 2. Chromatographic data obtained for all the tested compounds from MLC technique (k, log k_m) with the ODS-2 column and Brij 35 as the effluent component and on IAM (log $k_{w,IAM}$) and ODS (log $k_{w,ODS}$) columns; k_m—parameters calculated from Equation (2); R^2—coefficient of determination calculated for Equation (2).

No	k 0.075 M Brij 35	k 0.1 M Brij 35	k 0.125 M Brij 35	k 0.15 M Brij 35	log k_m	R^2	log $k_{w,IAM}$	log $k_{w,ODS}$
1	13.73	12.03	10.47	10.53	1.29	0.8851	0.76 [19]	1.59 [19]
2	25.51	21.45	18.25	17.34	1.68	0.9662	1.05 [19]	2.01 [19]
3	8.98	8.18	7.46	7.20	1.07	0.9725	0.65 [19]	1.42 [19]
4	39.84	31.65	24.39	22.52	2.28	0.9732	1.49 [19]	2.38 [19]
5	41.53	33.76	26.18	24.10	2.22	0.9754	1.39 [19]	2.32 [19]
6	60.81	46.76	34.01	29.82	2.82	0.9854	1.67 [19]	2.68 [19]
7	14.11	12.22	10.72	10.02	1.37	0.9862	0.94 [19]	1.92 [19]
8	6.50	5.97	5.52	5.28	0.92	0.9889	0.76 [19]	1.81 [19]
9	25.67	21.44	17.64	16.14	1.81	0.9870	1.25 [19]	2.16 [19]
10	5.89	5.40	5.04	4.79	0.88	0.9929	0.67 [19]	1.65 [19]
11	10.61	9.36	8.27	7.66	1.24	0.9947	0.88 [19]	1.86 [19]
12	36.90	29.09	22.17	19.83	2.52	0.9855	1.66 [19]	2.43 [19]
13	41.71	32.28	25.32	22.45	2.49	0.9911	1.58 [19]	2.40 [19]
14	59.61	44.28	32.68	28.56	2.70	0.9887	2.29 [19]	2.96 [19]
15	2.09	1.90	1.81	1.84	0.38	0.8467	0.48	1.21
16	4.49	4.04	3.81	3.75	0.74	0.9084	1.93	2.80
17	10.12	8.72	8.00	8.00	1.12	0.8604	1.73	2.62
18	9.94	8.48	7.53	7.75	1.12	0.8467	1.12	1.92
19	24.39	18.98	14.97	14.81	1.83	0.9117	3.36	3.24

Table 3. The established Quantitative Structure-Activity Relationships (n—number of observations, R^2—coefficient of determination, sd—standard deviation, F-value, p —probability value, VIF—variance inflation factor).

No of Equation	QSAR Equations	n	R^2	sd	F	p	VIF
(3)	$\log K_\mathrm{p} = -7.137(0.935) + 0.272(0.079)\log k_\mathrm{m} - 0.025(0.006)TPSA + 0.041(0.030)\alpha + 0.003(0.003)MW$	19	0.9593	0.108	83	0.000000	<4.4
(4)	$\log K_\mathrm{p} = -6.109(0.540) + 0.110(0.053)\log k_\mathrm{w,IAM} - 0.044(0.002)TPSA + 0.035(0.013)\alpha + 0.005(0.001)MW$	19	0.9677	0.096	106	0.000000	<2.7
(5)	$\log K_\mathrm{p} = -6.250(0.428) + 0.157(0.058)\log k_\mathrm{w,ODS} - 0.043(0.002)TPSA + 0.034(0.012)\alpha + 0.004(0.001)MW$	19	0.9723	0.089	123	0.000000	<2.3
(6)	$\log K_\mathrm{a,HSA} = 2.383(0.244) + 0.063(0.022)\log k_\mathrm{m} + 0.010(0.007)\alpha + 0.008(0.001)MW$	19	0.9368	0.064	75	0.000000	<1.3
(7)	$\log K_\mathrm{a,HSA} = 2.412(0.4220) + 0.018(0.043)\log k_\mathrm{w,IAM} + 0.008(0.010)\alpha + 0.008(0.001)MW$	19	0.9031	0.079	47	0.000000	<2.6
(8)	$\log K_\mathrm{a,HSA} = 2.356(0.3500) + 0.019(0.049)\log k_\mathrm{w,ODS} + 0.008(0.009)\alpha + 0.008(0.001)MW$	19	0.9028	0.079	46	0.000000	<2.1
(9)	$\log BB = -0.041(0.155) + 0.017(0.020)\log k_\mathrm{m} - 0.019(0.002)TPSA + 0.043(0.007)\alpha$	19	0.9554	0.048	108	0.000000	<2.6
(10)	$\log BB = 0.051(0.150) + 0.033(0.017)\log k_\mathrm{w,IAM} - 0.020(0.001)TPSA + 0.041(0.006)\alpha$	19	0.9630	0.043	130	0.000000	<1.8
(11)	$\log BB = -0.005(0.133) + 0.049(0.020)\log k_\mathrm{w,\,ODS} - 0.020(0.001)TPSA + 0.040(0.06)\alpha$	19	0.9673	0.041	148	0.000000	<1.8
(12)	$\text{Caco-2 E06} = 159.92(28.86) + 3.76(4.77)\log k_\mathrm{m} - 1.90(0.29)TPSA + 0.47(0.13)MW$	19	0.8799	9.723	37	0.000000	<2.5
(13)	$\text{Caco-2 E06} = 203.38(31.52) + 10.08(4.16)\log k_\mathrm{w,IAM} - 2.11(0.18)TPSA + 0.34(0.12)MW$	19	0.9101	8.413	51	0.000000	<2.2
(14)	$\text{Caco-2 E06} = 183.81(26.42) + 12.63(4.90)\log k_\mathrm{w,ODS} - 2.04(0.17)TPSA + 0.35(0.11)MW$	19	0.9133	8.260	53	0.000000	<2
(15)	$f_\mathrm{u,brain} = 0.739(0.147) - 0.014(0.024)\log k_\mathrm{m} + 0.012(0.001)TPSA - 0.003(0.001)MW$	19	0.9139	0.050	53	0.000000	<2.5
(16)	$f_\mathrm{u,brain} = 0.585(0.174) - 0.036(0.023)\log k_\mathrm{w,IAM} + 0.013(0.001)TPSA - 0.003(0.001)MW$	19	0.9243	0.046	61	0.000000	<2.2
(17)	$f_\mathrm{u,brain} = 0.643(0.144) - 0.050(0.027)\log k_\mathrm{w,ODS} + 0.012(0.001)TPSA - 0.003(0.001)MW$	19	0.9286	0.045	65	0.000000	<2
(18)	$\log Kp = -6.780(0.470) + 0.079(0.122)\log k_{0.1} - 0.043(0.005)TPSA + 0.042(0.017)\alpha + 0.006(0.001)MW$	19	0.9590	0.109	82	0.000000	<4.6
(19)	$\log K_\mathrm{a,HSA} = 2.292(0.255) + 0.106(0.043)\log k_{0.1} + 0.010(0.007)\alpha + 0.008(0.001)MW$	19	0.9300	0.067	67	0.000000	<1.2
(20)	$\log BB = -0.052(0.155) + 0.033(0.043)\log k_{0.1} - 0.019(0.002)TPSA + 0.042(0.007)\alpha$	19	0.9551	0.048	107	0.000000	<3
(21)	$\text{Caco-2 E06} = 153.28(26.64) + 13.46(8.48)\log k_{0.1} - 1.75(0.28)TPSA + 0.43(0.11)MW$	19	0.8929	9.182	42	0.000000	<2.3
(22)	$f_\mathrm{u,brain} = 0.762(0.142) - 0.038(0.045)\log k_{0.1} + 0.012(0.001)TPSA - 0.001(0.001)MW$	19	0.9158	0.049	55	0.000000	<2.3

2.2. Establishment of Quantitative Structure-Activity Relationships

In the QSARs methodology, we used the experimentally derived lipophilicity (chromatographic parameters: $\log k_m$, $\log k_{w,IAM}$, and $\log k_{w,ODS}$), structural (topological polar surface area *TPSA*, molecular weight *MW*), and electronic (polarizability α) descriptors as independent variables. These values were used to predict different pharmacokinetic parameters (dependent variables) evaluated for tested compounds (Table 2). Table 3 shows the quantitative structure-activity relationships (expressed as Equations (3)–(22)) that were established. The relationships were validated, and the results are presented in Table 4.

Table 4. Statistical parameters of Equation (3)–(22): PRESS—predicted residual sum of squares, MSE—mean square error, cv—cross validated.

Equation	Adjusted R^2	*PRESS*	*MSE*	R^2cv	*PRESS*cv	*MSE*cv
Equation (3)	0.9476	0.279	0.012	0.9593	0.279	0.012
Equation (4)	0.9585	0.220	0.009	0.9677	0.220	0.009
Equation (5)	0.9644	0.170	0.008	0.9368	0.093	0.004
Equation (6)	0.9241	0.093	0.004	0.9368	0.093	0.004
Equation (7)	0.8837	0.153	0.006	0.9031	0.153	0.006
Equation (8)	0.8834	0.147	0.006	0.9028	0.147	0.006
Equation (9)	0.9465	0.052	0.002	0.9554	0.052	0.002
Equation (10)	0.9556	0.043	0.002	0.9630	0.043	0.002
Equation (11)	0.9608	0.036	0.002	0.9673	0.036	0.002
Equation (12)	0.8559	2468	94.54	0.8799	2468	94.54
Equation (13)	0.8921	1827	70.8	0.9101	1827	66.54
Equation (14)	0.8960	1824	68.23	0.9133	1824	68.23
Equation (15)	0.8967	0.058	0.002	0.9139	0.058	0.002
Equation (16)	0.9091	0.049	0.002	0.9243	0.049	0.002
Equation (17)	0.9143	0.044	0.002	0.9286	0.044	0.002
Equation (18)	0.9473	0.284	0.012	0.9590	0.248	0.012
Equation (19)	0.9160	0.111	0.005	0.9300	0.111	0.005
Equation (20)	0.9462	0.054	0.002	0.9551	0.054	0.002
Equation (21)	0.8715	2311	84.32	0.8929	2311	84.32
Equation (22)	0.8989	0.058	0.002	0.9158	0.058	0.002

The statistical parameters allowed us to evaluate the derived QSAR equations as being very good. There were no significant cross-correlations between the parameters that characterized the substances, i.e., the values of the variance inflation factor (*VIF*) were significantly lower than 5. The diagrams presented in Figure 1A show the standard coefficients of Equations (3)–(5) established for the $\log K_p$ as an example. The remaining diagrams are presented in Figures S1A–S4A in the Supplementary Material. They explain both the direction and strength of the impact of a given structural descriptor on the calculated biological parameter. The correlations shown in Figure 1B illustrate the relationships between the $\log K_p$ values calculated with the ACD/Percepta software (actual response) and those predicted by the QSARs models (calculated response) that were developed (Equations (3)–(5)). The remaining diagrams are presented in Figures S1B–S4B in the Supplementary Material. The applicability domain (AD) of the developed regression models was also evaluated and visualized as the Williams plots (Figure 1C and Figures S1C–S4C in the Supplementary Material). AD is a theoretical region in physicochemical space (the response and chemical structure space) for which a QSAR model should make predictions with a given reliability. The warning leverage limits ($h^* = 0.789$ and 0.632) were calculated using the following equation:

$$h^* = \frac{3(k+1)}{n} \tag{23}$$

where k is a number of descriptors used in the MLR model and n is the number of compounds in the dataset. The Williams plot can be used for graphical detection of outliers ($h > h^*$) [25]. The results proved that the obtained models are valid within the domain in which they were developed.
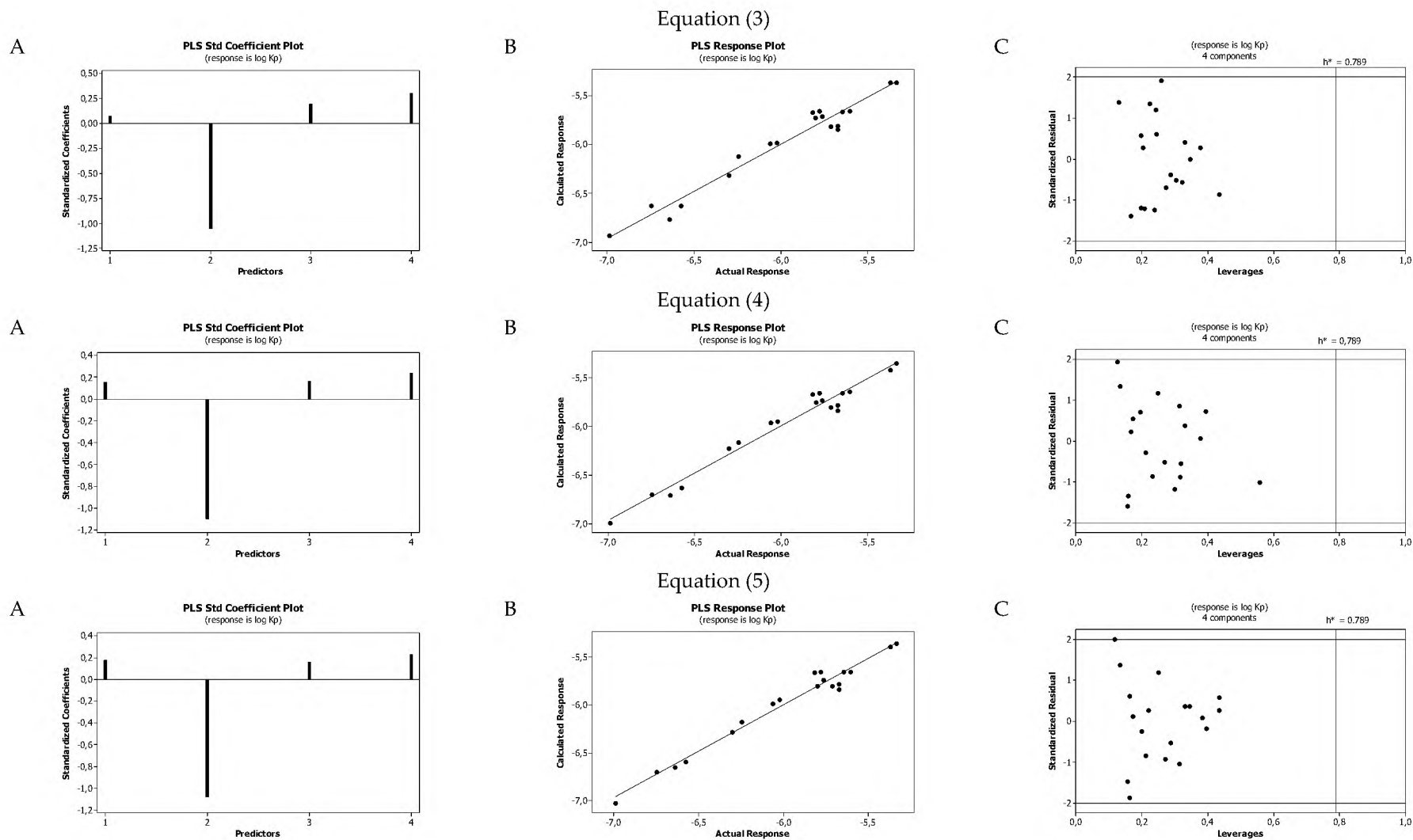
Figure 1. Standardized coefficients (**A**), the correlation between actual (ACD/Percepta) and predicted (Equations (3)–(5)) log K_p parameters (**B**), and the Williams plots of Equations (3)–(5) (**C**).

2.3. Assessing the Risk of Undesired Effects

Many of potential molecular pharmaceutics cannot be subjected to clinical trials on humans due to the risk of serious adverse effects. Hence, in silico tools such as the OSIRIS Property Explorer (http://www.organic-chemistry.org/prog/peo/ accessed on 25 March 2021), recommended by Food and Drug Administration, are helpful in qualitative prediction of serious adverse side effects in the early stage of the drug development process. For all the investigated compounds 1-19 considered as potential anticancer agents, no risk of mutagenicity, tumorigenicity, and irritating effects were predicted. In addition, no risk (for compounds 1-14) or medium risk (for compounds 15-19) of reproductive effects was found. This is as expected due to the lack of "genotoxicophore" fragments in the tested molecules. The results are shown in Table S1 in the Supplementary Material.

3. Discussion

The lipophilic properties of compounds increase their binding to human serum albumin and to the lipids contained in biological membranes. In the investigations, the lipophilic properties of our compounds were described based on their chromatographic retention (log k_m, log $k_{w,ODS}$, and log $k_{w,IAM}$), and the positive effect of these parameters on the log K_p, log $K_{a,HSA}$, log BB, Caco-2, and $f_{u,brain}$ values was obtained (Figure 1, Figures S1–S4 in the Supplementary Material). Taking into account the standardized coefficients, the lipophilicity had a similar, moderate impact on the above parameters. The same effect was found for α, i.e., the polarizability of the molecules. This parameter increased the strength of the van der Waals interactions between the solutes and the albumin or lipids molecules [26]. Thus, the polarizability of the molecule increased the values of log K_p, log $K_{a,HSA}$, and log BB. Polarizability increased the values of log K_p and log $K_{a,HSA}$ similarly to or slightly more than lipophilicity. In the case of log BB, polarizability seemed to be the dominant positive factor. We observed no effect of polarizability on the values of Caco-2 and $f_{u,brain}$ (Equations (12)–(17)). The positive effect of molecular weight (MW) on the values of log K_p, log K_{aHSA}, and Caco-2 could be explained by the partition mechanism of the permeation of the tested substances through biological membranes as well as human serum albumin. Similarly, Abraham et al. [27] obtained the positive effect of molecular size on the permeability through the skin. This relationship is a reflection of the correlation between the size of the molecules and lipophilicity. In addition, molecular size has a negative correlation with diffusivity in biomembranes, confirming that the effects of partitioning are more dominant than the effects of diffusion [28].

The polar molecular surface area (PSA) is defined as the surface area occupied by the nitrogen and oxygen atoms and the polar hydrogens bonded to these heteroatoms. The penetration of substances through biological barriers decreases when the hydrophilic part of its surface increases. PSA has been used extensively as a molecular descriptor in the studies of drug transport properties, such as intestinal absorption [29], BBB penetration [30], and membrane permeability [28,31,32]. Topological surface area ($TPSA$), a convenient measure of the polar surface area, was introduced by Ertl et al. [33] as the effect of the additive fragment method and is extremely popular in medicinal chemistry [34] for predicting the properties of ADME. In our research, we observed a significant negative impact of $TPSA$ on log K_p, log BB, and the Caco-2 parameters (Equations (3)–(5) and (9)–(14)). The increase of the polar surface area decreased the permeability through the skin, permeation of the blood–brain barrier, and intestinal permeability.

The factors that increase the substances that bind to serum albumin and lipids cause a simultaneous reduction of the unbound fraction in the brain, $f_{u,brain}$. The equations derived in our studies (Equations (15)–(17)), Figure S4 in the Supplementary Material) show that $f_{u,brain}$ decreased with increasing lipophilicity and molecular weight (MW) but increased with the hydrophilicity ($TPSA$) of the compound. Polarizability had a negligible effect on the $f_{u,brain}$ values.

In RPLC, the standard lipophilicity descriptors are the log k_w parameters evaluated for water (buffer) as the mobile phase. In the case of micellar chromatography, the log k_m

values were used (Equation (2)), corresponding to the mobile phase without any "free" surfactant molecules. In general, the determination of these parameters is time-consuming and requires multiple measurements using different mobile phases. Nevertheless, the quantities determined in this way are more reliable and similar to the partitioning parameter, log *P*. Frequently, in practice, the chromatographic parameters measured with mobile phases that contain an organic modifier can also be used to evaluate lipophilicity. Most often, experimental data are used that were measured with columns imitating biological systems, such as artificial membranes, immobilized cholesterol, and others. In our studies, we obtained very good linear correlations between the log *k* values obtained in MLC for mobile phases with different concentrations of Brij 35, i.e., 0.15 mol/L, 0.10 mol/L, 0.125 mol/L, and 0.075 mol/L (Table 2). The correlation factors of these relationships were in the range of 0.902–0.942. Therefore, we decided to use the log *k* parameters measured in one micellar effluent to derive the quantitative structure-activity relationships. We chose the values measured in the mobile phase composed of 0.1 mol/L of surfactant Brij 35, i.e., log $k_{0.1}$. For this mobile phase, the retention of individual substances was not too high (log *k* values in the range of 0.279–1.67). At the same time, the flow of effluent through the column was not associated with high pressure. Appropriate equations (Equations (18)–(22)) and statistics are presented in Tables 3 and 4. In the statistical evaluation, these equations were similar and almost as good as those derived for the log k_m, log $k_{w,IAM}$, and log $k_{w,ODS}$ parameters. The results indicate the effectivity of micellar chromatography and its predictive ability in assessing the properties of bioactive substances. This technique also provided the advantage of being able to mimic biopartitioning systems. On the basis of the chromatographic measurements performed in one system with a micellar mobile phase, our results show that there is a high probability that the pharmacokinetic properties of the tested compounds can be predicted accurately.

4. Materials and Methods

4.1. Reagents and Materials

Isopropanol, acetonitrile (HPLC grade), and polyoxyethylene lauryl ether (Brij 35) (for synthesis) were supplied from Merck (Lublin, Poland). Citric acid and Na_2HPO_4 (both pure) were purchased from POCh (Lublin, Poland). Deionized water was produced using the Direct-Q3 UV system (Millipore, Warsaw, Poland).

4.2. Instrumental

Shimadzu Vp (Shimadzu, Izabelin, Polska) liquid chromatographic system was used in HPLC measurements. It was equipped with an LC 10AT pump, SPD 10A UV–Vis detector, SCL 10A system controller, CTO-10 AS chromatographic oven, and Rheodyne injector valve with a 20 µL loop. As the stationary phases, 3 different revered-phase materials were applied: Spherisorb ODS-2 column, 125 × 4 mm i.d., 5 µm (Merck, Lublin, Poland), Regis IAM.PC.DD2 column, 100 × 4.6 mm i.d., 10 µm (Morton Grove, Illinois, USA), and Purosphere RP-18e column, 125 × 4 mm i.d., 5 µm (Merck, Lublin, Poland).

4.3. Chromatographic Conditions

In the MLC technique with an ODS-2 column, buffered Brij 35 mixtures (0.15; 0.125, 0.10, and 0.075 mol/L) with 7% (*v/v*) addition of isopropanol were used as mobile phases. The buffer was prepared from 0.01 mol/L solutions of Na_2HPO_4 and citric acid, and the pH 7.4 value was fixed before mixing with an organic modifier. The flow rate was 1 mL/min. Buffered acetonitrile mixtures were used as effluents with the IAM column. Acetonitrile concentration, expressed as a volume fraction, was changed in the range of 0.2–0.5, with the constant step of 0.1. The flow rate was 1.3 mL/min. Acetonitrile concentration was changed in the range of 0.3–0.6 with the RP-18e column, with the constant step of 0.1 and flow rate of 0.1 mL/min. As solutes tested there were used 19 newly designed structurally related compounds. Samples were dissolved in acetonitrile c.a. 0.005 mg/mL. The compounds were detected under UV light at λ_{max} 254 nm. All measurements were carried out at a

constant temperature (25 °C). The dead time values were measured from non-retained compound (e.g., sodium chloride) peaks. All reported k values are the average of at least 3 independent measurements.

4.4. In Silico Calculations

Molecular weight (*MW*), topological polar surface area (*TPSA*) and polarizability (α) of the tested compounds (as independent variables), as well as pharmacokinetic parameters characterizing their distribution between the blood and brain (log *BB*), fraction unbounded in brain ($f_{u,brain}$), water–skin permeation (K_p), binding to human plasma proteins (log $K_{a,HSA}$), intestinal permeability (Caco-2) (as dependent variables), and the logarithms of n-octanol/water partition coefficient (logs *P*), were evaluated by ACD/Percepta software (Łódź, Poland). In this software, the logs *P* and pharmacokinetic descriptors are calculated from Abraham solvation parameters (i.e., the McGowan volume, polarizability/dipolarity, hydrogen bond basicity (accepting ability) and hydrogen bond acidity (donating ability), excess molar refraction, etc.), according to the concept of LSERs (linear solvation energy relationships) [35].

The risk of adverse effects of the investigated compounds was evaluated by the OSIRIS software, which is available online: http://www.organic-chemistry.org/prog/peo/ (accessed on 25 March 2021). This in silico tool uses the final datasets from the Registry of Toxic Effects of Chemical Substances (RTECS) database containing 7504 mutagenic, 2841 tumorigenic, 2372 irritant, and 3570 reproductive effective substances, as well as 3343 pharmaceutics as a control set. The qualitative prediction result encoded in green, yellow, and red indicates no risk, medium risk, and high risk of undesired effects, respectively.

4.5. Statistical Analysis

Linear regression (LR), multiple linear regression (MLR), and leave-one-out cross validation (LOOcv) were done employing the statistical software Minitab 16 (Minitab Inc., State College, PA, USA).

5. Conclusions

Two-dimensional QSAR methodology was successful in modeling pharmacokinetic properties, i.e., the distribution between the blood and brain (log *BB*), the unbounded fraction in the brain ($f_{u,brain}$), water–skin permeation (log K_p), binding to human plasma proteins (log $K_{a,HSA}$), and intestinal permeability (Caco-2) of fused azaisocytosine-containing congeners. Various liquid chromatography techniques were used to characterize all the title compounds regarded as promising drug candidates. Micellar parameters (log k_m) and log k_w values measured on an artificial membrane (IAM) and on an end-capped ODS column were compared as lipophilicity descriptors and applied in the QSARs methodology. Apart from the chromatography-derived lipophilicity, the quantitative structure-activity relationships included both structural and electronic descriptors related to drug-like properties, i.e., topological polar surface area, molecular weight, and polarizability of the investigated molecules. All the derived QSAR equations were evaluated statistically and validated as being very good. It should be noted that the QSAR models that were developed revealed a high predictive ability and therefore provided reliable predictions in modeling the pharmacokinetic properties of the title molecules. All models used for prediction of the dependent solute property linked the retention parameters on MLC, IAM, and ODS with additional molecular descriptors related to drug-like properties. All the dependent pharmacokinetic properties obtained on the basis of QSAR equations were compared with those calculated in silico and were statistically validated as being very good. Applicability domains of the developed regression models were evaluated and visualized. The investigations highlight the significance and possibilities of combined chromatographic techniques and QSARs methods in modeling important pharmacokinetic properties of our structurally related small molecules and reducing unethical animal testing. The micellar liquid chromatography technique made it possible to achieve a significant reduction in the time and cost of the

experiments and also reduced the consumption of organic reagents. The results presented in this study will be particularly useful in further, more extensive in vivo research of the title compounds that are being considered as potential drugs.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijms22084257/s1. Figure S1. Standardized coefficients (A), the correlation between actual (ACD/Percepta) and predicted (Equations (6)–(8)) log $K_{a,HSA}$ parameters (B), and the Williams plots of Equations (6)–(8) (C). Figure S2. Standardized coefficients (A), the correlation between actual (ACD/Percepta) and predicted (Equations (9)–(11)) log BB parameters (B), and the Williams plots of Equations (9)–(11) (C). Figure S3. Standardized coefficients (A), the correlation between actual (ACD/Percepta) and predicted (Equations (12)–(14)) Caco-2 parameters (B), and the Williams plots of Equations (12)–(14) (C). Figure S4. Standardized coefficients (A), the correlation between actual (ACD/Percepta) and predicted (Equations (15)–(17)) $f_{u,brain}$ parameters (B), and the Williams plots of Equations (15)–(17) (C). Table S1. Risk assessment of adverse side effects by OSIRIS Property Explorer for the investigated compounds 1-19.

Author Contributions: Conceptualization, M.J.; Methodology, M.J., A.M.; Investigation, M.J., A.M.; Formal analysis, M.J.; Funding acquisition, M.S., K.S.; Resources, M.J., M.S., K.S.; Writing—Original Draft, M.J., M.S., K.S.; Writing—Review & Editing, M.J., M.S., K.S. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

Sample Availability: Samples of all the investigated fused azaisocytosine-containing congeners 1-19 are available from the authors.

References

1. Hansch, C. Quantitative approach to biochemical structure-activity relationships. *Accounts Chem. Res.* **1969**, *2*, 232–239. [CrossRef]
2. Hansch, C. Quantitative structure-activity relationships and the unnamed science. *Accounts Chem. Res.* **1993**, *26*, 147–153. [CrossRef]
3. Kaliszan, R. *Quantitative Structure-Chromatographic Retention Relationships*; John Wiley & Sons: New York, NY, USA, 1987.
4. Héberger, K. Quantitative structure–(chromatographic) retention relationships. *J. Chromatogr. A* **2007**, *1158*, 273–305. [CrossRef] [PubMed]
5. Valkó, K.L. Lipophilicity and biomimetic properties measured by HPLC to support drug discovery. *J. Pharm. Biomed. Anal.* **2016**, *130*, 35–54. [CrossRef]
6. Andrić, F.; Bajusz, D.; Rácz, A.; Šegan, S.; Héberger, K. Multivariate assessment of lipophilicity scales-computational and re-versed phase thin-layer chromatographic indices. *J. Pharm. Biomed. Anal.* **2016**, *127*, 81–93. [CrossRef] [PubMed]
7. Janicka, M.; Sztanke, M.; Sztanke, K. Predicting the Blood-Brain Barrier Permeability of New Drug-Like Compounds via HPLC with Various Stationary Phases. *Molecules* **2020**, *25*, 487. [CrossRef] [PubMed]
8. Ayouni, L.; Cazorla, G.; Chaillou, D.; Herbreteau, B.; Rudaz, S.; Lantéri, P.; Carrupt, P.-A. Fast Determination of Lipophilicity by HPLC. *Chromatographia* **2005**, *62*, 251–255. [CrossRef]
9. Barbato, F.V.; Cirocco, L.; Grumetto, M.; La Rotonda, I. Comparison between immobilized artificial membrane (IAM) HPLC data and lipophilicity in n-octanol for quinolone antibacterial agents. *Eur. J. Pharm. Sci.* **2007**, *31*, 288–297. [CrossRef] [PubMed]
10. Kempińska, D.; Chmiel, T.; Kot-Wasik, A.; Mróz, A.; Mazerska, Z.; Namieśnik, J. State of the art and prospects of methods for determination of lipophilicity of chemical compounds. *TrAC Trends Anal. Chem.* **2019**, *113*, 54–73. [CrossRef]
11. Testa, B.; Crivori, P.; Reist, M.; Carrupt, P.-A. The influence of lipophilicity on the pharmacokinetic behavior of drugs: Concepts and examples. *Persp. Drug Disc. Des.* **2000**, *19*, 179–211. [CrossRef]
12. Ciura, K.; Dziomba, S. Application of separation methods for in vitro prediction of blood–brain barrier permeability—The state of the art. *J. Pharm. Biomed. Anal.* **2020**, *177*, 112891. [CrossRef]
13. Rutkowska, E.; Pajak, K.; Jóźwiak, K. Lipophilicity–methods of determination and its role in medicinal chemistry. *Acta. Pol. Pharm. Drug Res.* **2013**, *70*, 3–18.
14. Rybińska-Fryca, A.; Sosnowska, A.; Puzyn, T. Representation of the Structure—A Key Point of Building QSAR/QSPR Models for Ionic Liquids. *Materials* **2020**, *13*, 2500. [CrossRef]
15. Ruiz-Ángel, M.; Carda-Broch, S.; Torres-Lapasió, J.; García-Álvarez-Coque, M. Retention mechanisms in micellar liquid chromatography. *J. Chromatogr. A* **2009**, *1216*, 1798–1814. [CrossRef]

16. Escuder-Gilabert, L.; Molero-Monfort, M.; Villanueva-Camañas, R.M.; Sagrado, S.; Medina-Hernández, M.J. Potential of biopartitioning micellar chromatography as an in vitro technique for predicting drug penetration across the blood-brain barrier. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2004**, *807*, 193–201. [CrossRef]
17. Anastas, P.T.; Warner, J.C. *Green Chemistry: Theory and Practice*; Oxford University Press: New York, NY, USA, 1998.
18. Pliśka, V.; Testa, B.; van de Waterbeemd, H. *Lipophilicity in Drug Action and Toxicology*; VCH Inc.: Weinheim, Germany, 1996.
19. Sztanke, M.; Rzymowska, J.; Janicka, M.; Sztanke, K. Two novel classes of fused azaisocytosine-containing congeners as promising drug candidates: Design, synthesis as well as in vitro, ex vivo and in silico studies. *Bioorg. Chem.* **2020**, *95*, 103480. [CrossRef]
20. Sztanke, K.; Rzymowska, J.; Niemczyk, M.; Dybała, I.; Kozioł, A.E. Synthesis, crystal structure and anticancer activity of novel derivatives of ethyl 1-(4-oxo-8-aryl-4,6,7,8-tetrahydroimidazo[2,1-c][1,2,4]triazin-3-yl)formate. *Eur. J. Med. Chem.* **2006**, *41*, 539–547. [CrossRef] [PubMed]
21. Sztanke, M.; Tuzimski, T.; Janicka, M.; Sztanke, K. Structure–retention behaviour of biologically active fused 1,2,4-triazinones – Correlation with in silico molecular properties. *Eur. J. Pharm. Sci.* **2015**, *68*, 114–126. [CrossRef] [PubMed]
22. Sztanke, M.; Rzymowska, J.; Sztanke, K. Anticancer active trifluoromethylated fused triazinones are safe for early-life stages of zebrafish (*Danio rerio*) and reveal a proapoptotic action. *J. Enzyme Inhib. Med. Chem.* **2021**, *36*, 336–344. [CrossRef] [PubMed]
23. García-Alvarez-Coque, M.C.; Ruiz-Angel, M.J. Micellar liquid chromatography. *Encycl. Anal. Sci.* **2019**, 133–142.
24. Foley, J.P. Critical compilation of solute-micelle binding constants and related parameters from micellar liquid chromatographic measurements. *Anal. Chim. Acta* **1990**, *231*, 237–247. [CrossRef]
25. Gramatica, P. On the development and Validation of QSAR Models. In *Computational Toxicology*; Reisfeld, B., Mayeno, A.N., Eds.; Springer Humana Press: Berlin, Germany, 2013; Volume II.
26. Tandon, H.; Ranjan, R.; Chakraborty, T.; Suhag, V. Polarizability: A promising descriptor to study chemical-biological interactions. *Mol. Divers.* **2021**, *25*, 249–262. [CrossRef]
27. Abraham, M.H.; Chadha, H.S.; Mitchell, R.C. The Factors that Influence Skin Penetration of Solutes*. *J. Pharm. Pharmacol.* **2011**, *47*, 8–16. [CrossRef]
28. Yamashita, F.; Hashida, M. Mechanistic and empirical modeling of skin permeation of drugs. *Adv. Drug Deliv. Rev.* **2003**, *55*, 1185–1199. [CrossRef]
29. Li, S.F.; He, H.D.; Parthiban, L.J.; Yin, H.Q.; Serajuddin, A.T.M. IV-IVC considerations in the development of immediate-release oral dosage form. *J. Pharm. Sci.* **2005**, *94*, 1396–1417. [CrossRef]
30. Strazielle, N.; Ghersi-Egea, J.F. Factors affecting delivery of antiviral drugs to the brain. *Rev. Med. Virol.* **2005**, *15*, 105–133. [CrossRef] [PubMed]
31. Potts, R.O.; Guy, R.H. Predicting Skin Permeability. *Pharm. Res.* **1992**, *9*, 663–669. [CrossRef] [PubMed]
32. Chi, C.-T.; Lee, M.-H.; Weng, C.-F.; Leong, M.K. In silico prediction of PAMPA effective permeability using a two-QSAR approach. *Int. J. Mol. Sci.* **2019**, *20*, 3170. [CrossRef]
33. Ertl, P.; Rohde, B.; Selzer, P. Fast Calculation of Molecular Polar Surface Area as a Sum of Fragment-Based Contributions and Its Application to the Prediction of Drug Transport Properties. *J. Med. Chem.* **2000**, *43*, 3714–3717. [CrossRef]
34. Prasanna, S.; Doerksen, R.J. Topological Surface Area: A Useful Descriptor in 2D-QSAR. *Curr. Med. Chem.* **2009**, *16*, 21–41. [CrossRef] [PubMed]
35. Vitha, M.; Carr, P.W. The chemical interpretation and practice of linear solvation energy relationships in chromatography. *J. Chromatogr. A* **2006**, *1126*, 143–194. [CrossRef] [PubMed]

Article

Quantitative Retention (Structure)–Activity Relationships in Predicting the Pharmaceutical and Toxic Properties of Potential Pesticides

Małgorzata Janicka [1],* and Anna Śliwińska [2]

[1] Department of Physical Chemistry, Faculty of Chemistry, Institute of Chemical Science,
    Maria Curie-Skłodowska University, 20-031 Lublin, Poland
[2] Doctoral School of Quantitative and Natural Sciences, Maria Curie-Skłodowska University,
    20-031 Lublin, Poland; aea.sliwinska@gmail.com
*   Correspondence: malgorzata.janicka@mail.umcs.pl

Abstract: The micellar liquid chromatography technique and quantitative retention (structure)–activity relationships method were used to predict properties of carbamic and phenoxyacetic acids derivatives, newly synthesized in our laboratory and considered as potential pesticides. Important properties of the test substances characterizing their potential significance as pesticides as well as threats to humans were considered: the volume of distribution, the unbonded fractions, the blood–brain distribution, the rate of skin and cell permeation, the dermal absorption, the binding to human serum albumin, partitioning between water and plants' cuticles, and the lethal dose. Pharmacokinetic and toxicity parameters were predicted as functions of the solutes' lipophilicities and the number of hydrogen bond donors, the number of hydrogen bond acceptors, and the number of rotatable bonds. The equations that were derived were evaluated statistically and cross-validated. Important features of the molecular structure influencing the properties of the tested substances were indicated. The QSAR models that were developed had high predictive ability and high reliability in modeling the properties of the molecules that were tested. The investigations highlighted the applicability of combined chromatographic technique and QS(R)ARs in modeling the important properties of potential pesticides and reducing unethical animal testing.

Keywords: lipophilicity; micellar chromatography; pesticides; QRARs; QSARs

1. Introduction

Pesticides are very important substances in the modern world. They help to increase the efficiency of agricultural production and food processing by protecting crops against bacteria, fungi and molds, insects, rodents, and weeds. Since pesticides are used in the countryside, in forests, and in cities, people are constantly exposed to contact with these substances in their diets [1–3]. Although scientists do not have a full understanding of the health effects of pesticide residues, there is no doubt that the use of these substances must be limited and controlled. As new pesticide-active compounds are developed, it is vitally important to be able to predict their properties, their pharmacokinetics, and toxicities at the earliest stage of the research. Although modern science makes it possible to predict in silico the properties of substances only on the basis of their molecular structure, the results of these calculations rarely are highly reliable, and they generally require experimental verification. To avoid highly unethical and costly animal testing, alternative techniques in combination with in silico modeling can be used to predict the properties of drug-like or pesticide-like compounds in screening [4,5].

Reversed-phase liquid chromatography (RPLC), both planar and column, is a technique commonly used to assess the lipophilic properties of bioactive organic substances [6–8]. Chromatography with stationary phases that imitate biological partitioning systems, such

as an artificial membrane, phases with immobilized lipids, albumin, cholesterol, ceramides, or liposomes, allows the prediction of the lipophilic properties [6–10] as well as the behaviors of solutes in real biological systems, such as bound to serum albumin, skin permeation, blood–brain barrier permeability, intestinal absorption, the concentration of unbound form in the blood, and others [11–16]. Similar possibilities are offered by micellar liquid chromatography (MLC) using surfactants as components of the mobile phase. MLC is a mode of conventional RPLC using a surfactant solution above the critical micellization concentration (*cmc*) in the mobile phase. Under these conditions, the micelles form the so-called micellar pseudophase in the bulk phase. The surrounding bulk water or aqueous-organic mixture contains surfactant monomers in a concentration approximately equal to the *cmc*. Surfactant monomers modify the surface phase as a result of the hydrophobic interactions between the tail of the surfactant and the alkyl chain. Molecular interactions present in this system, i.e., solute association with the polar head of the surfactant, solute penetration into the micelle core, and solute interactions with adsorbed surfactant and alkyl chains, affect retention by three different equilibria, which are (1) the solute distribution between the micelle (micellar pseudophase) and the bulk phase, (2) the solute partition between the stationary phase modified by the surfactant and the bulk phase, and (3) the direct transfer of solute molecules between the surfactant-modified surface and the micelle [17–21].

Several theories have been developed that describe the retention in MLC, i.e., the effect of the concentration of the surfactant in the effluent on the retention of the solute. Foley's equation [22] is best known in lipophilicity studies, and according to Foley, the following relationship exists between the retention parameter, k, and the concentration of the surfactant in the effluent:

$$\frac{1}{k} = \frac{1}{k_m} + \frac{K_{AM}}{k_m}[M]$$

(1)

where $[M]$ is the total concentration of surfactant in the mobile phase minus *cmc*, K_{AM} is the constant that describes solute–micelle binding, and k_m is the solute retention parameter at zero micellar concentration, i.e., at surfactant monomer concentration equal to *cmc*. The K_{AM} and k_m parameters can be evaluated from the slope and intercept of experimental $1/k$ vs. $[M]$ relationships. Equation (1) describes a linear dependence with decreasing retention as the micelle concentration increases. This equation is valid for aqueous solutions of a surfactant or mobile phases with the same concentrations of the organic modifier. The micellar retention parameter, log k_m, is considered analogous to the log k_w value evaluated in RPLC. Thus, this parameter is considered a lipophilicity descriptor, and Equation (1) is a simple way to achieve the indirect determination of the lipophilic properties of compounds. It is postulated that retention in micellar chromatography depends on the hydrophobic (lipophilic), electronic, and steric features of the compounds in a similar way as many pharmacokinetic phenomena. An additional similarity is indicated by the fact that the phospholipids, cholesterol, fatty acids, and triglycerides that are present in the extracellular and intracellular fluids also form micelles with proteins.

In the studies, 15 carbamic and phenoxyacetic acids derivatives (Table 1), newly synthesized in our laboratory, and considered potential pesticides, were investigated using the column micellar liquid chromatography technique. As solutes lipophilicity descriptors there were applied k_m and K_{AM} values, calculated form Equation (1) [23]. Pharmacokinetic and toxicity parameters were predicted as functions of the solutes' lipophilicities (QRARs model) or lipophilicity and the number of hydrogen bond donors (*HBD*), the number of hydrogen bond acceptors (*HBA*), and the number of rotatable bonds (*NRB*) (QSARs model).

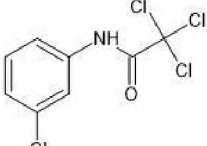**Table 1.** Structures and chromatographic parameters [23] of tested compounds.
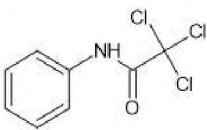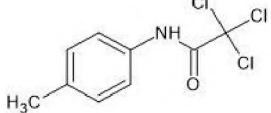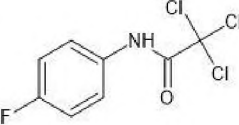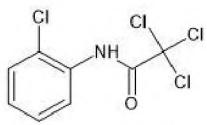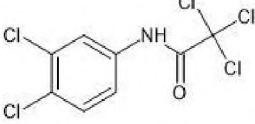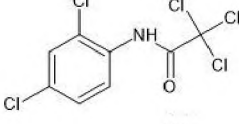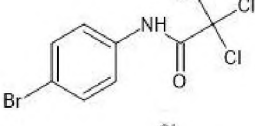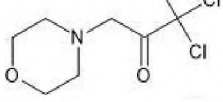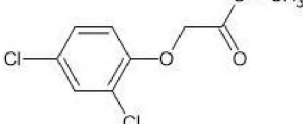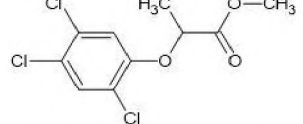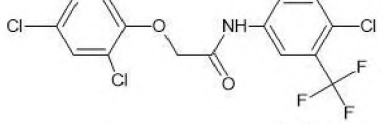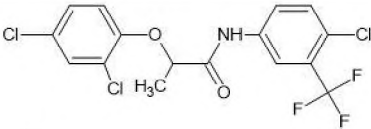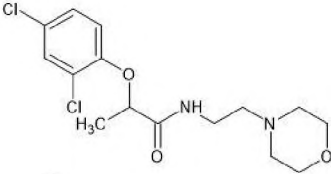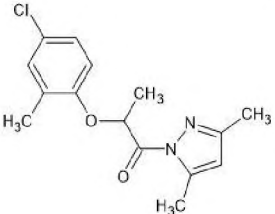
No	Group	Structure	log k_m	log K_{AM}
1			2.00	1.76
2			2.46	2.13
3			2.10	1.80
4			2.72	2.31
5	I		2.40	1.98
6			3.20	2.60
7			2.96	2.39
8			2.92	2.46
9			0.40	−0.48
10			2.00	1.76
11			2.95	2.39
12			3.90	3.40

Table 1. *Cont.*

No	Group	Structure	log k_m	log K_{AM}
13	II		4.30	4.00
14			1.60	1.23
15			2.71	2.28

2. Results

The retention parameters k_m and K_{AM} values are presented in Table 1. The relationship between the values was checked, and the following rectilinear relationship was obtained:

$$\log k_m = 0.585(0.102) + 0.932(0.044) \log K_{AM} \qquad (2)$$

$n = 15$; $s = 0.1612$; $R = 0.9861$; $R_{adj.} = 0.9849$; $F = 456$; $p = 0.000000$.

The above confirms that both micellar parameters, i.e., $\log k_m$ and $\log K_{AM}$, could be used as alternative descriptors of the lipophilicities of compounds.

The physicochemical, pharmacokinetic, and toxicity parameters of the compounds (Table 2) are as follows: the logarithm of the partition coefficient ($\log P$) in the *n*-octanol/water system, the number of hydrogen bond donors (*HBD*), acceptors (*HBA*), and rotatable bonds (*NRB*), molar weight (*MW*), topological polar surface area (*TPSA*) [24], the volume of distribution in the body (V_d) [25], the fraction unbonded in a brain ($f_{u, brain}$), in plasma ($f_{u, plasma}$) [26], and pharmacokinetic parameters describing blood–brain distribution ($\log BB$) [26–29], the rate of permeation from aqueous solutions through skin ($\log K_p$) [30,31], skin–water partition coefficient ($\log K_{sc}$) describing dermal absorption from aqueous solutions [32,33], the rate of permeation through cell ($\log K_{w/cell}$) [34], partitioning between water and serum albumin ($\log P_{w/HSA}$), and binding to human serum albumin ($\log K_{HSA}$) [10,35–37], partitioning between water and plant' cuticles ($\log P_{w/pc}$) [38], and the dose causing the death of 50% of the group of mice tested after oral administration (LD_{50}) [39,40]. These parameters describe important properties of the test substances and provide information about their potential applications as pesticides as well as potential threats to humans [41,42].

Table 2. Physicochemical, pharmacokinetic, and toxicity parameters of tested compounds.

Nr	$\log P$	HBD	HBA	NRB	MW [g/mol]	TPSA [A^2]	V_d [L/kg]	$f_{\mathrm{u, plasma}}$	$f_{\mathrm{u, brain}}$	$\log BB$	$\log K_\mathrm{p}$	$\log K_\mathrm{sc}$	$\log K_{\mathrm{w/cell}}$	$\log P_{\mathrm{w/HSA}}$	$\log K_{\mathrm{HSA}}$	$\log P_{\mathrm{w/pc}}$	LD_{50} [mg/kg]
1	2.285	1	2	2	238.50	29.10	2.1	0.0750	0.06	−0.218	−5.095	1.713	−4.988	0.280	4.58	2.513	2600
2	2.937	1	2	2	252.52	29.10	2.3	0.0830	0.04	−0.026	−4.747	1.996	−4.833	0.554	4.60	3.106	2000
3	2.309	1	2	2	256.49	29.10	2.1	0.1000	0.06	−0.275	−5.075	1.717	−5.164	0.253	4.86	2.504	1800
4	2.971	1	2	2	272.94	29.10	2.4	0.0370	0.02	−0.098	−4.710	2.100	−5.071	0.694	4.70	3.254	1900
5	2.810	1	2	2	272.94	29.10	2.1	0.0510	0.03	−0.110	−4.836	1.989	−4.951	0.576	5.11	3.077	2000
6	3.755	1	2	2	307.39	29.10	2.8	0.0140	0.01	0.056	−4.244	2.529	−4.944	1.132	4.97	4.092	1800
7	3.488	1	2	2	307.39	29.10	2.4	0.0320	0.01	−0.011	−4.434	2.390	−5.020	0.993	5.53	3.847	2300
8	2.980	1	2	2	317.39	29.10	2.4	0.0540	0.02	−0.094	−4.818	2.134	−5.197	0.804	4.93	3.331	1400
9	1.345	0	3	3	232.49	29.54	1.5	0.3100	0.30	−0.225	−5.538	1.097	−3.947	−0.492	4.17	1.522	1600
10	2.917	0	3	4	235.06	35.53	1.9	0.0920	0.07	0.263	−4.431	1.923	−3.279	0.313	4.36	3.132	550
11	4.067	0	3	4	283.53	35.53	2.1	0.0610	0.02	0.513	−3.731	2.541	−3.081	0.904	4.94	4.400	580
12	4.936	1	3	5	398.59	38.33	3.8	0.0030	0.01	0.227	−3.741	3.107	−5.275	1.642	5.35	5.420	780
13	5.386	1	3	5	412.62	38.33	4.0	0.0029	0.01	0.345	−3.504	3.308	−5.213	1.823	5.39	5.858	720
14	2.600	1	5	5	347.24	50.80	1.6	0.2400	0.12	−0.229	−5.761	1.608	−5.822	0.200	3.98	2.765	840
15	3.807	0	4	3	292.76	44.12	2.1	0.0690	0.02	0.448	−4.540	2.386	−3.999	1.017	4.17	4.240	750

3. Discussion

Based on the anticipated in silico parameters (Table 2), it is important to note that the substances that were tested met the basic requirements formulated by Lipinski as the "rule of five" [43,44]: lipophilicities expressed as log *P* values are not greater than 5 (with the exception of substance no. 13); molecular weights are not greater than 500 g/mole; numbers of hydrogen bonds acceptors are not greater than 10, and the numbers of hydrogen bond donors are not greater than 5. In addition, the topological polar surface areas are below 90 Å^2, and the number of rotatable bonds is in the range of 2–5. The compounds have moderate in silico predicted V_d values ($V_d < 7$ L/kg), indicating that they do not accumulate to a significant extent in fat tissue. The highest values of V_d were observed for compounds no. 12 and no. 13, the most lipophilic among all of the compounds that were tested. The values of log K_{HSA}, which describe binding to human serum albumins, were in the range of 3.98–5.53, whereas the log $P_{w/HSA}$ parameters that characterized the solute partitioning between water and serum albumin were in the range of 0.200–1.823. Xenobiotics bound to plasma proteins are not active because they are not able to cross membranes and permeate the site of action nor bind to receptors. The binding to serum albumins affects the concentration of the unbonded forms of the substance in serum, and small values of free fractions are preferable in order to prevent possible side effects.

Parameters that have negative values of log *BB* or values close to zero (in the range of −0.275 to −0.513) suggest that the compounds that were tested will not be able to penetrate into the brain, and neurotoxicity will be diminished. Of course, low levels of penetration into the brain are desirable. Compounds no. 12 and 13 had positive values of log *BB*, and they had the lowest unbonded fractions in the brain ($f_{u, brain}$). Compounds no. 12 and 13, which had the highest log K_{HSA} and log $P_{w/HSA}$ values, also had the lowest unbonded fractions in plasma ($f_{u, plasma}$). According to the parameters calculated *in silico*, these substances are characterized by the highest rate of permeation through the skin (log K_p), dermal absorption (log K_{sc}), and partition between water and plant cuticle (log $K_{w/cell}$), as well as the lowest rate of permeation through cells (log $P_{w/pc}$). Substance no. 14 is the most toxic, poorly bound with albumins, and its concentration in the unbound form in the brain and serum was the highest among the substances that were tested, even though the value of log *BB* was negative.

When considering different parameters (e.g., chromatographic retention) as lipophilicity descriptors, they should be compared with the log *P* values that describe solute partitioning between *n*-octanol and water. In our studies, the relationships between the chromatographic and partition coefficient log *P* are presented in Figure 1. In both cases, i.e., for log k_m and log K_{AM}, the separate relationships for group I (carbamic acid derivatives) and group II (phenoxyacetic acid derivatives) were obtained with very good linearity ($R >> 0.8$). They confirm both micellar parameters as lipophilicity descriptors of the compounds that were tested.

(A)

(B)
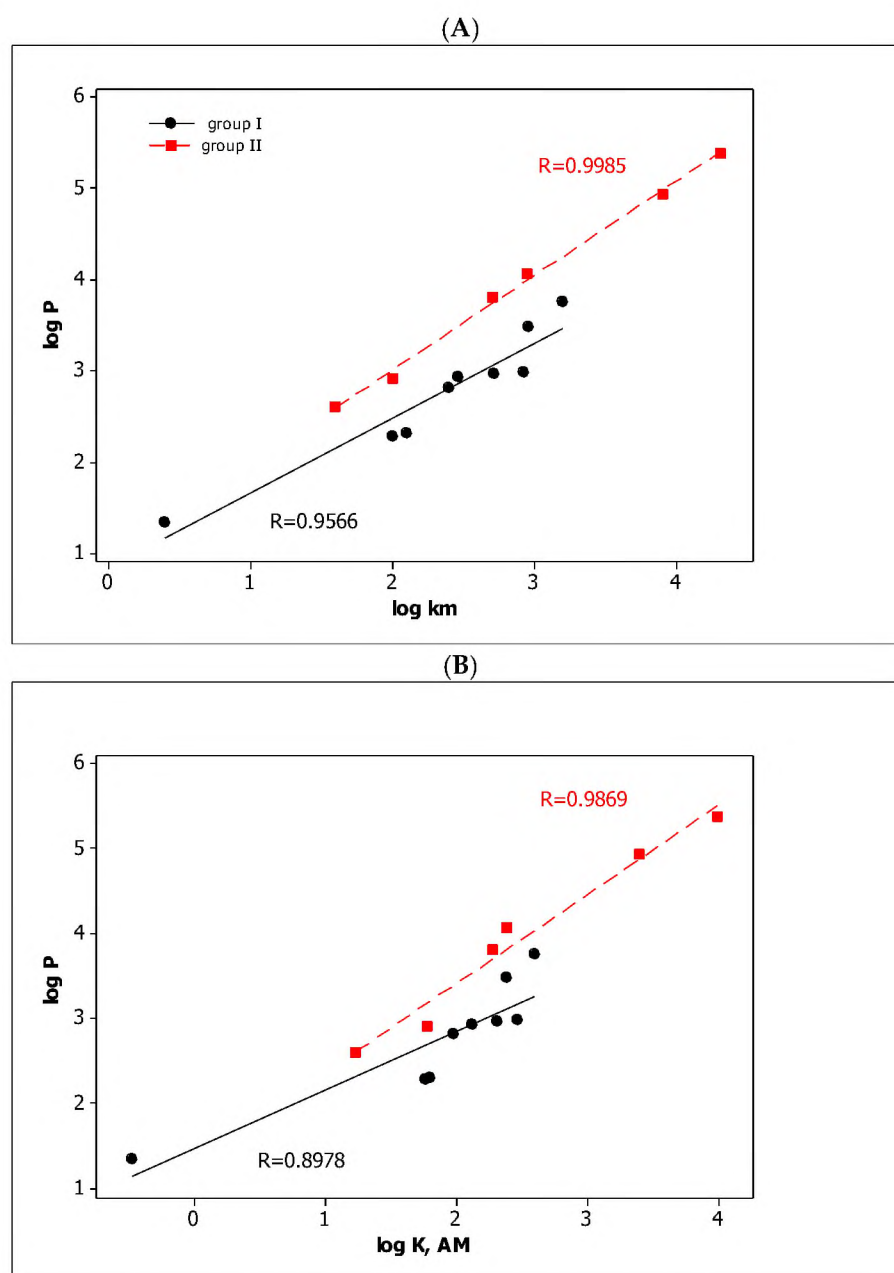
Figure 1. The log P vs. log k_m (**A**) and log P vs. log K_{AM} (**B**) relationships obtained for tested compounds.

3.1. QRARs

In QRARs, it is desirable to have the methodology relationships between solute retention and the biological activity of the compounds. In our investigations, we obtained the linear relationships between the micellar parameters (log k_m and log K_{AM}) and the other parameters, i.e., log K_{sc} and log $P_{w, HSA}$ (Figure 2) and V_d, log $P_{w/pc}$, $f_{u, brain}$, and $f_{u, plasma}$ (Figure 3) with very good quality ($R \gg 0.8$). The straight lines in Figure 2A,B show a clear increase in dermal absorption and partitioning in the water–human serum albumin system of tested compounds with an increase in their lipophilicity. It should be noted that the lipophilic properties of the tested substances, based on the in silico log P parameters, are in the range of 5.386–1.345. The increase in lipophilicity in the parabolic function affects the volume of distribution (V_d) and absorption by the plant cuticle (log $P_{w/pc}$) as well as the unbonded fraction in the brain ($f_{u, brain}$) and plasma ($f_{u, plasma}$) (Figure 3). Although V_d and log $P_{w/pc}$ increase with lipophilicity, the other parameters, i.e., $f_{u, plasma}$ and $f_{u, brain}$,

decrease. The graphs suggest the existence of the optimal range of lipophilicity of the substance, for which the volume of distribution and the absorption through the epidermis are the highest, and the unbonded fractions in plasma and in the brain are the lowest. Figures 2 and 3 also indicate that lipophilicity is the dominant factor that influences (1) the absorption of the test substances through the skin and epidermis, (2) the distribution of water–albumin, (3) the size of the unbound fraction in the plasma and the brain, and (4) the volume of the distribution.
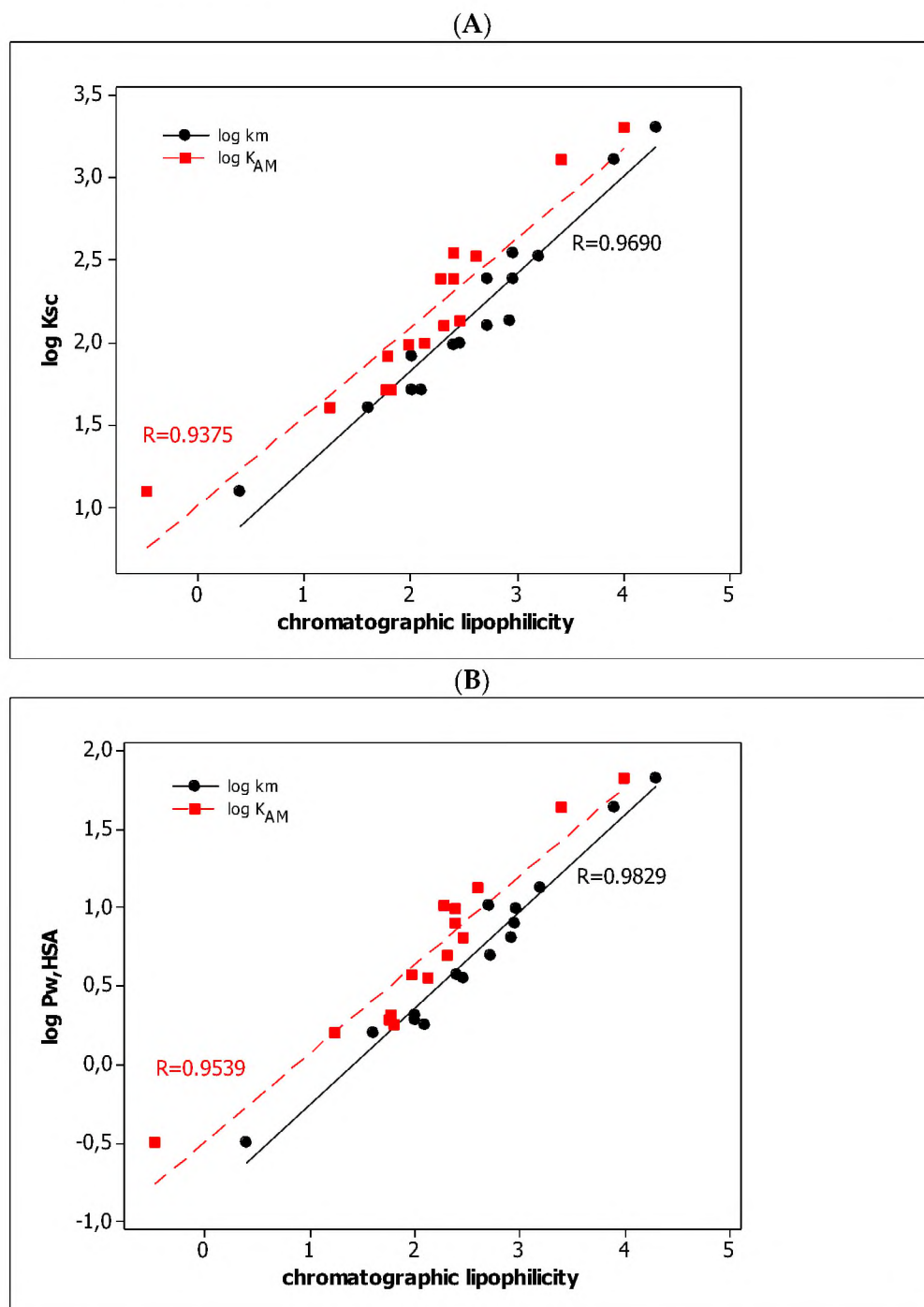
Figure 2. The linear relationships between log K_{sc} (**A**) and log $P_{w, HSA}$ (**B**) and chromatographic parameters (log k_m and log K_{AM}).

(A)

(B)
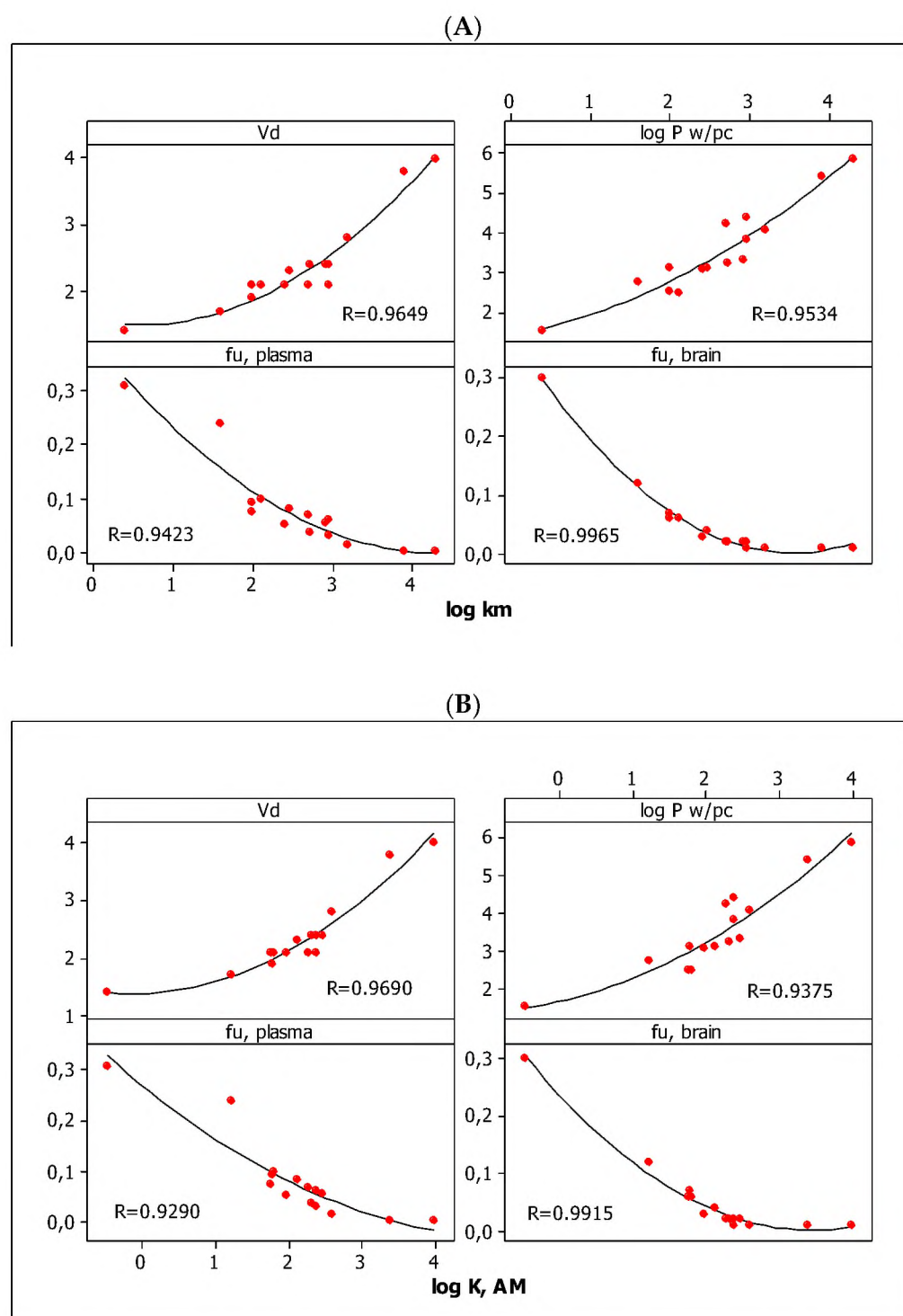
Figure 3. The parabolic relationships between pharmacokinetic parameters and log k_m (**A**) and log K_{AM} (**B**) values.

3.2. QSARs

In the QSARs methodology, we used the experimentally-derived lipophilicities (micellar parameters log k_m and log K_{AM}), and the numbers of hydrogen bond donors (*HBD*), acceptors (*HBA*), and rotatable bonds (*NRB*) as independent variables. These values were used to predict the dependent variables, i.e., log K_p, log $K_{w/cell}$, log K_{HSA}, log *BB*, and LD_{50}. Table 3 shows the quantitative structure–activity relationships (expressed as Equations (3)–(12)) that were established. The equations were cross-validated (LOO), and

all of the calculated statistics are summarized in Table 3 and presented graphically in Figures 4–8 as PLS-standardized coefficients (A), the response plots (B), and standardized residuals vs. leverages (C). The statistical parameters allowed us to positively evaluate the derived QSAR equations. There were no significant cross-correlations between the independent variables, and the values of the variance inflation factor (*VIF*) were significantly lower than 5. The diagrams presented in Figures 4A, 5A, 6A, 7A and 8A show the standard coefficients of Equations (3)–(12), and they explain the direction and the strength of the impact of a given descriptor on the calculated parameters. The correlations shown in Figures 4B, 5B, 6B, 7B and 8B illustrate the relationships between the actual response (values obtained from ACD/Percepta software) and those predicted by the established QSAR models (calculated response). The applicability domains (*AD*) of the developed regression models were also evaluated and are visualized as the Williams plots (Figures 4C, 5C, 6C, 7C and 8C). *AD* is a theoretical region in physicochemical space (the response and chemical structure space) for which a model should make predictions with a given reliability [45]. The warning leverage limits ($h^* = 1.0$) were calculated using the following equation:

Table 3. The established quantitative structure–activity relationships: n—number of observations, R—coefficient of determination, $R_{adj.}$—adjusted coefficient of determination, sd—standard deviation, F-value, p—probability value, VIF—variance inflation factor, $PRESS$—predicted residual sum of squares, MSE—mean square error, cv—cross-validated.

No.	Equation
(3)	$\log K_p = -5.329(0.156) + 0.597(0.041)\log k_m - 0.669(0.075)HBD - 0.345(0.061)HBA + 0.197(0.044)NRB$ $n = 15; R = 0.9893; R_{adj.} = 0.9850; sd = 0.110; PRESS = 0.276; MSE = 0.012; F = 116; p < 0.0001; VIF < 3.6; PRESS_{cv} = 0.276; MSE_{cv} = 0.012$
(4)	$\log K_p = -4.900(0.218) + 0.552(0.060)\log K_{AM} - 0.714(0.118)HBD - 0.378(0.092)HBA + 0.213(0.067)NRB$ $n = 15; R = 0.9747; R_{adj.} = 0.9644; sd = 0.169; PRESS = 0.610; MSE = 0.0286; F = 48; p < 0.0001; VIF < 3.5; PRESS_{cv} = 0.610; MSE_{cv} = 0.0286$
(5)	$\log K_{w/cell} = -2.773(0.295) + 0.062(0.078)\log k_m - 1.846(0.143)HBD - 0.368(0.115)HBA + 0.076(0.083)NRB$ $n = 15; R = 0.9740; R_{adj} = 0.9634; sd = 0.209; PRESS = 4.064; MSE = 0.0435; F = 47; p < 0.0001; VIF < 3.6; PRESS_{cv} = 4.064; MSE_{cv} = 0.0435$
(6)	$\log K_{w/cell} = -2.762(0.262) + 0.079(0.073)\log K_{AM} - 1.867(0.142)HBD - 0.356(0.111)HBA + 0.064(0.081)NRB$ $n = 15; R = 0.9753; R_{adj} = 0.9652; sd = 0.203; PRESS = 4.889; MSE = 0.0414; F = 49; p < 0.0001; VIF < 3.5; PRESS_{cv} = 4.889; MSE_{cv} = 0.0400$
(7)	$\log K_{HSA} = 4.619(0.350) + 0.251(0.093)\log k_m + 0.106(0.170)HBD - 0.381(0.136)HBA + 0.150(0.100)NRB$ $n = 15; R = 0.8953; R_{adj} = 0.8498; sd = 0.248; PRESS = 1.078; MSE = 0.0615; F = 11; p < 0.0001; VIF < 3.6; PRESS_{cv} = 1.078; MSE_{cv} = 0.0615$
(8)	$\log K_{HSA} = 4.843(0.351) + 0.204(0.097)\log K_{AM} + 0.109(0.190)HBD - 0.415(0.148)HBA + 0.175(0.108)NRB$ $n = 15; R = 0.8722; R_{adj} = 0.8155; sd = 0.272; PRESS = 1.326; MSE = 0.0741; F = 8; p < 0.0001; VIF < 3.5; PRESS_{cv} = 1.326; MSE_{cv} = 0.0741$
(9)	$\log BB = -0.375(0.078) + 0.254(0.021)\log k_m - 0.454(0.038)HBD + 0.041(0.030)HBA - 0.006(0.023)NRB$ $n = 15; R = 0.9836; R_{adj} = 0.9770; sd = 0.055; PRESS = 0.090; MSE = 0.0031; F = 75; p < 0.0001; VIF < 3.6; PRESS_{cv} = 0.090; MSE_{cv} = 0.0031$
(10)	$\log BB = -0.205(0.073) + 0.242(0.020)\log K_{AM} - 0.479(0.039)HBD + 0.032(0.031)HBA - 0.003(0.022)NRB$ $n = 15; R = 0.9831; R_{adj} = 0.9762; sd = 0.056; PRESS = 0.069; MSE = 0,0032; F = 72; p < 0.0001; VIF < 3,5; PRESS_{cv} = 0,069; MSE_{cv} = 0.0032$
(11)	$LD_{50} = 2870(411) - 253(109)\log k_m + 650(199)HBD - 194(160)HBA - 245(116)NRB$ $n = 15; R = 0.9330; R_{adj} = 0.9048; sd = 291; PRESS = 2078292; MSE = 84613; F = 17; p < 0.0001; VIF < 3.6; PRESS_{cv} = 2078292; MSE_{cv} = 76083$
(12)	$LD_{50} = 2714(367) - 251(102)\log K_{AM} + 682(192)HBD - 192(155)HBA - 242(113)NRB$ $n = 15; R = 0.9358; R_{adj} = 0.9088; sd = 285; PRESS = 2017628; MSE = 81170; F = 18; p < 0.0001; VIF < 3.5; PRESS_{cv} = 2017628; MSE_{cv} = 74652$

$$h^* = \frac{3(k+1)}{n} \tag{13}$$

where k is the number of descriptors used in the MLR model, and n is the number of compounds in the data set. The Williams plot can be used for graphical detection of outliers ($h > h^*$).

The results proved that the models obtained are valid within the domain in which they were developed. The results obtained in our studies indicate the positive effect of solute lipophilicity on the skin (log K_p) (Figures 4 and 5) and cell permeation (log $K_{w/cell}$) (Figures 6 and 7) from water, binding affinity to human serum albumin (log K_{HSA}) (Figures 8 and 9), concentration in the brain (log BB) (Figures 10 and 11), and toxicity in mice (the decrease in LD_{50}) (Figures 12 and 13). Lipophilicity is a dominant factor for log K_p, log K_{HSA}, log BB, and LD_{50}. The rates of cell permeation are strongly retarded by solute hydrogen bond acidity and rather less so by hydrogen bond basicity (Figures 6 and 7). The same effects of the compounds' acidity and basicity on skin permeation were observed (Figures 4 and 5). The number of hydrogen bond donors (*HBD*) also strongly reduces the substance permeation through the blood–brain barrier (Figures 10 and 11) and increases the value of the lethal dose (Figures 12 and 13). The values of LD_{50} decrease and the toxicity of the solutes increase with the number of hydrogen bond acceptors. Binding to human serum albumin is strongly related to (decreased) hydrogen bond basicity (*HBA*) and much less dependent (increased) on its acidity (*HBD*) (Figures 8 and 9).

Solute flexibility, as described by the *NRB* values, strongly increases the rate of dermal absorption (Figures 4 and 5) and binding to human serum albumin (Figures 8 and 9). It also reduces the LD_{50} value, i.e., increases the toxicity of the substance (Figures 12 and 13). *NRB* has a slightly positive effect on cell permeation (Figures 6 and 7). Hydrogen bond basicity and solute flexibility practically do not affect the penetration of substances through the blood–brain barrier (Figures 10 and 11).

When analyzing the results, substances no. 10–15 (phenoxyacetic acid derivatives) should be indicated as the most toxic for mice, i.e., having the lowest lethal dose after oral administration. These substances are more lipophilic among those tested (log *P* values are in the range of 2.6–5.386, with smaller *HBD* (*HBD* $\leq$ 1), and greater *HBA* (*HBA* $\geq$ 3) values, and they have the greatest number of rotatable bonds (*NRB* > 3). They also have a higher concentration in the brain; with the exception of compound no. 14, all of the log *BB* values were greater than 0.

Summarizing the results, substances no. 12 and no. 13 can be indicated as the most interesting among those that were tested. They are the most toxic, but they are also highly bound to plasma albumin, and their free fractions in plasma and the brain are the lowest. The magnitudes of the distribution are acceptable, as they were for all of the substances that were tested. On the basis of the results that were obtained, it can be concluded that they can be considered promising pesticides as well as subjects for further, more detailed research.
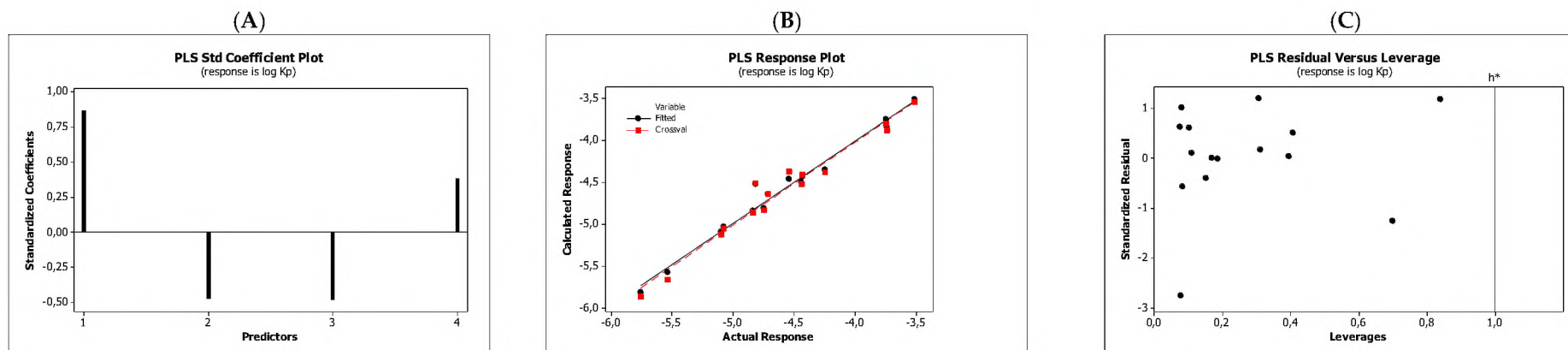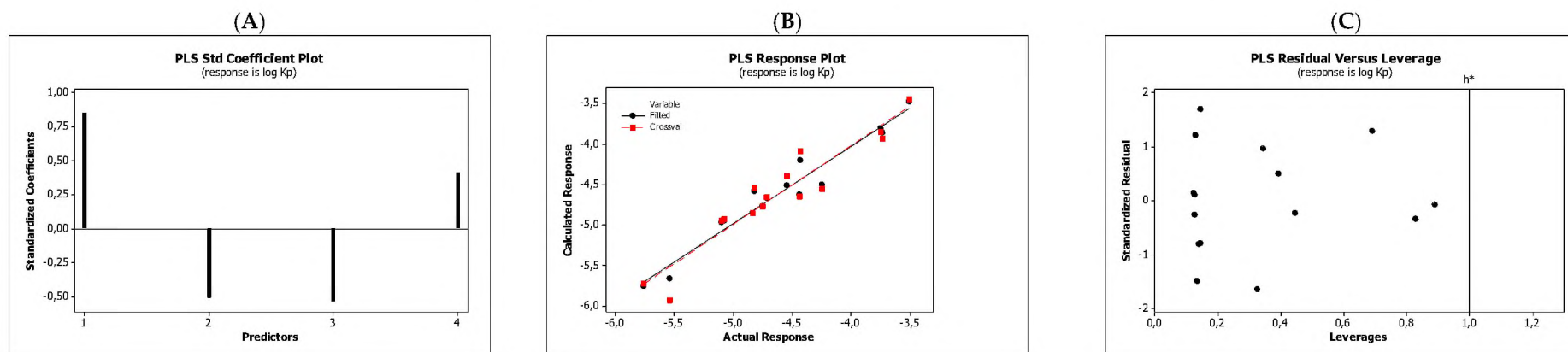
Figure 4. Standardized coefficients (**A**), the correlation between actual and predicted log K_P parameters (**B**), and the Williams plots (**C**) of Equation (3).
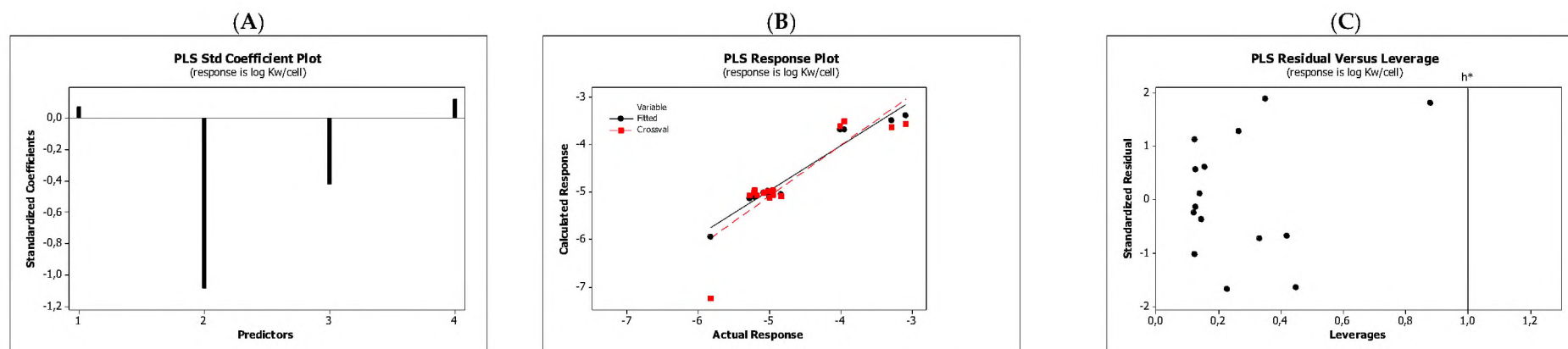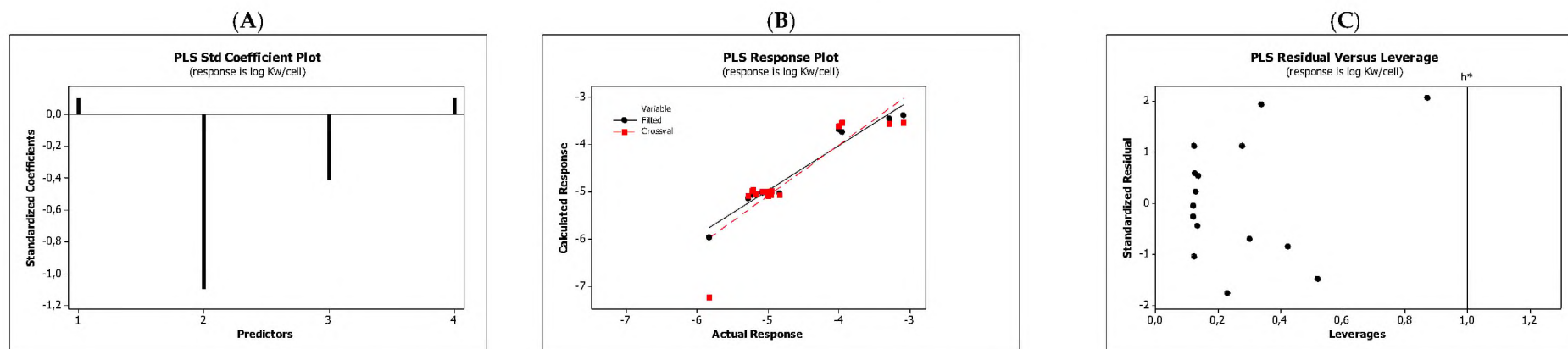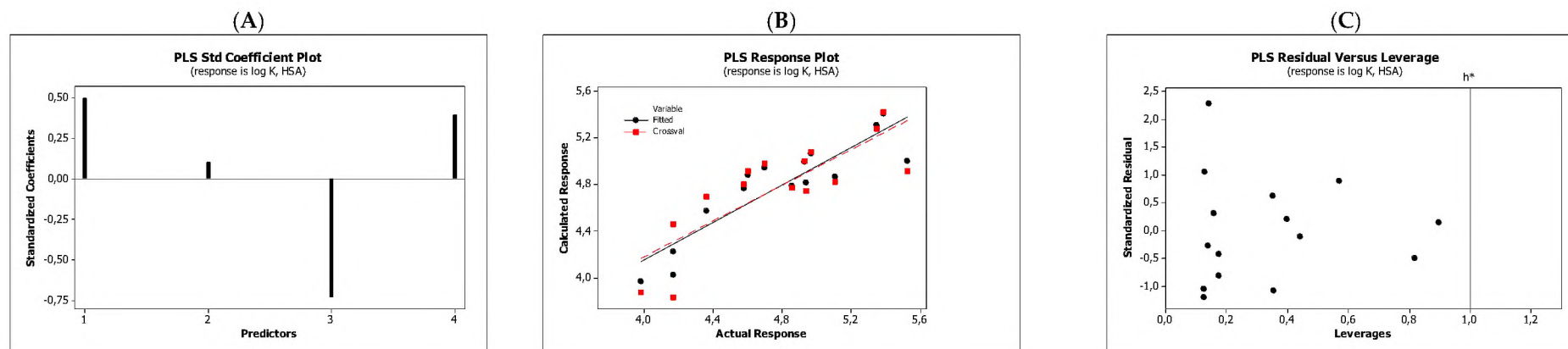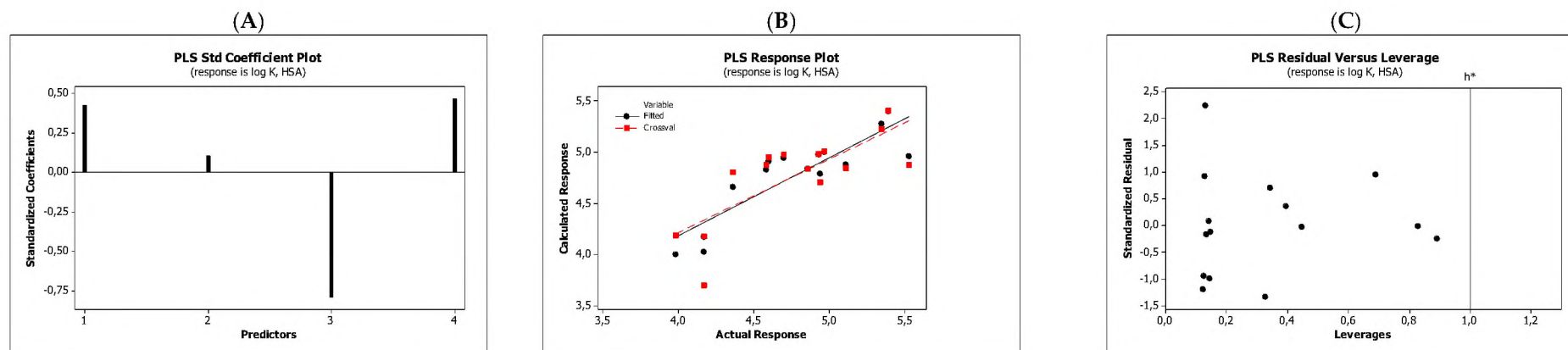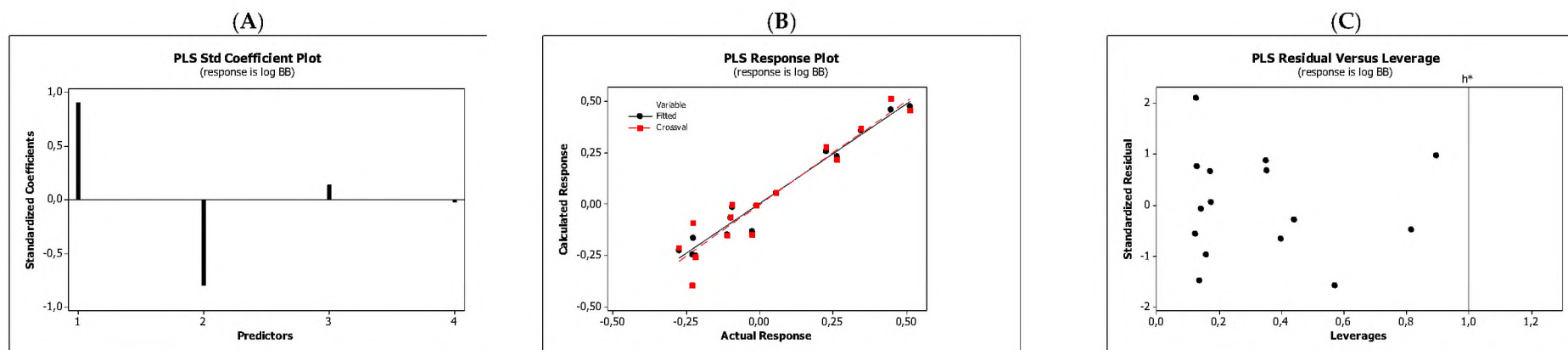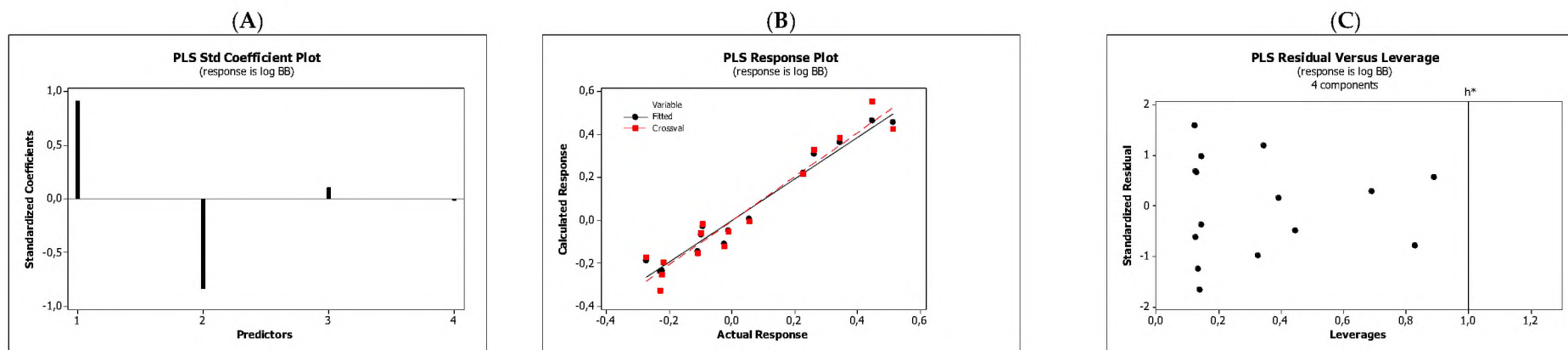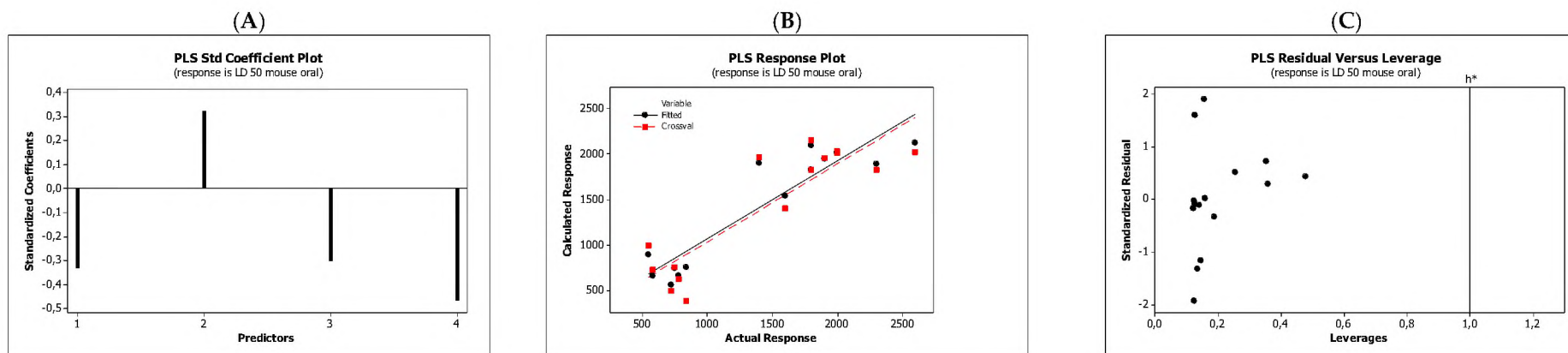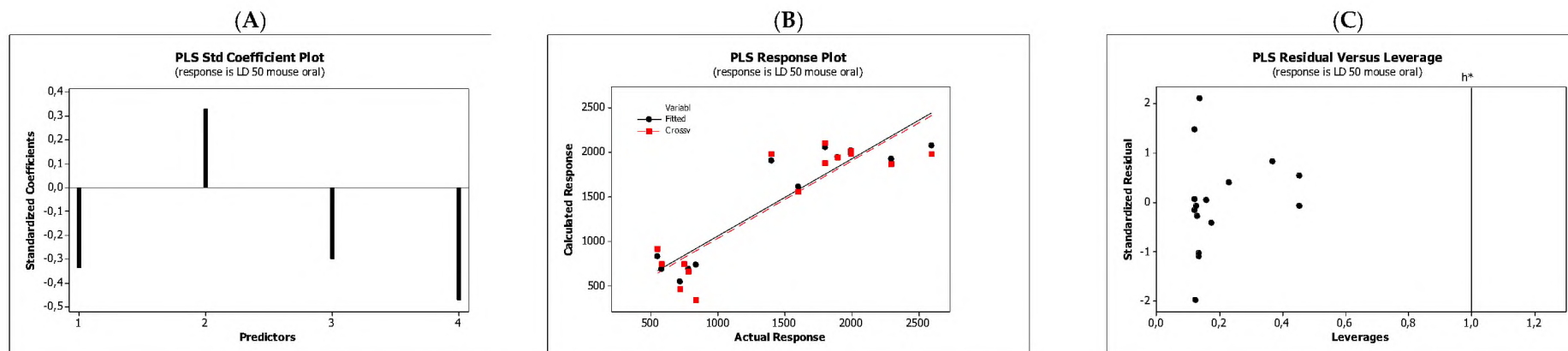

Figure 5. Standardized coefficients (**A**), the correlation between actual and predicted log K_P parameters (**B**), and the Williams plots (**C**) of Equation (4).

Figure 6. Standardized coefficients (**A**), the correlation between actual and predicted log $K_{\mathrm{w/cell}}$ parameters (**B**), and the Williams plots (**C**) of Equation (5).

Figure 7. Standardized coefficients (**A**), the correlation between actual and predicted log $K_{\mathrm{w/cell}}$ parameters (**B**), and the Williams plots (**C**) of Equation (6).

Figure 8. Standardized coefficients (**A**), the correlation between actual and predicted log $K_{a, HSA}$ parameters (**B**), and the Williams plots (**C**) of Equation (7).

Figure 9. Standardized coefficients (**A**), the correlation between actual and predicted log $K_{a, HSA}$ parameters (**B**), and the Williams plots (**C**) of Equation (8).

Figure 10. Standardized coefficients (**A**), the correlation between actual and predicted log *BB* parameters (**B**), and the Williams plots (**C**) of Equation (9).

Figure 11. Standardized coefficients (**A**), the correlation between actual and predicted log *BB* parameters (**B**), and the Williams plots (**C**) of Equation (10).

Figure 12. Standardized coefficients (**A**), the correlation between actual and predicted LD_{50} mouse oral parameters (**B**), and the Williams plots (**C**) of Equation (11).

Figure 13. Standardized coefficients (**A**), the correlation between actual and predicted LD_{50} mouse oral parameters (**B**), and the Williams plots (**C**) of Equation (12).

4. Materials and Methods

4.1. Chromatographic Measurements

Potential pesticides, i.e., carbamic (group I) and phenoxyacetic (group II) acids derivatives (Table 1), synthesized in our laboratory, were investigated using the micellar liquid chromatography technique. Previously, we reported [23] the chromatographic results that were obtained by the HPLC technique using a Purospher RP-8e column, four mobile phases composed of a buffer (pH = 7.4), four different sodium dodecyl sulfate (SDS) concentrations (i.e., 0.04, 0.06, 0.08, and 0.10 mol L^{-1}), and the same 20% (v/v) addition of acetonitrile.

4.2. In Silico Parameters

The physicochemical, structural, pharmacokinetic, and toxicity parameters of the compounds that were tested were calculated from their molecular structures using ACD/Percepta software, version 1994-2012 (ACD/Labs, Advanced Chemistry Development, Inc., Toronto, ON, Canada) (Table 2).

4.3. Statistics

Linear regression (LR), multiple linear regression (MLR), partial last squares (PLS), and leave-one-out cross-validation (LOO) were conducted using the statistical software Minitab 16.2.4.0, version 1991-2004 (Minitab Inc., State College, PA, USA).

5. Conclusions

QRARs and QSAR methodologies were successful in modeling the pharmacokinetic properties and toxicities of 15 newly synthesized compounds considered as potential pesticides. The micellar liquid chromatography technique was used to determine the lipophilicity descriptors (log k_m and log K_{AM}) of the compounds. In the QSAR method, log k_m and log K_{AM} parameters, *HBD*, *HBA*, and *NRB* were applied as independent values. All of the equations that were derived were evaluated statistically as being very good. The QSAR models that were developed had high predictive ability and high reliability in modeling the properties of the molecules that were tested. The investigations highlighted the significance and possibilities of combined chromatographic techniques and QR(S)ARs in modeling the important properties of potential pesticides and reducing unethical animal testing.

References

1. Li, Q.; Lu, Y.; Wang, P.; Wang, T.; Suriyanarayanan, S.; Liang, R.; Baninla, Y.; Khan, K. Distribution, source, and risk of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in urban and rural soils around the Yellow and Bohai Seas, China. *Environ. Pollut.* **2018**, *239*, 233–241. [CrossRef] [PubMed]
2. Syafrudin, M.; Kristanti, R.A.; Yuniarto, A.; Hadibarata, T.; Rhee, J.; Al-onazi, W.A.; Algarni, T.S.; Almarri, A.H.; Al-Mohaimeed, A.M. Pesticides in Drinking Water—A Review. *Int. J. Environ. Res. Public Health* **2021**, *18*, 468. [CrossRef] [PubMed]
3. Umetsu, N.; Shirai, Y. Development of novel pesticides in the 21st century. *J. Pestic. Sci.* **2020**, *45*, 54–74. [CrossRef]
4. Cimmino, A.; Zonno, M.C.; Andolfi, A.; Troise, C.; Motta, A.; Vurro, M.; Evidente, A. Agropyrenol, a phytotoxic fungal metabolite, and its derivatives: A structure−activity relationship study. *J. Agric. Food Chem.* **2013**, *61*, 1779−1783. [CrossRef]

5. Toropov, A.A.; Toropova, A.P.; Marzo, M.; Dorne, J.L.; Georgiadis, N.; Benfenati, E. QSAR models for predicting acute toxicity of pesticides in rainbow trout using the CORAL software and EFSA's OpenFoodTox database. *Environ. Tox. Pharm.* **2017**, *53*, 158–163. [CrossRef] [PubMed]

6. Kaliszan, R. QSRR Quantitative structure−(chromatographic) retention relationships. *Chem. Rev.* **2007**, *107*, 3212−3246. [CrossRef] [PubMed]

7. Kempińska, D.; Chmiel, T.; Kot-Wasik, A.; Mróz, A.; Mazerska, Z.; Namieśnik, J. State of the art and prospects of methods for determination of lipophilicity of chemical compounds. *Trends Anal. Chem.* **2019**, *113*, 54–73. [CrossRef]

8. Héberger, K. Quantitative structure-(chromatographic) retention relationships. *J. Chromatogr. A* **2007**, *1158*, 273–305. [CrossRef]

9. Andrić, F.; Héberger, K. Towards better understanding of lipophilicity: Assessment of in silico and chromatographic log P measures for pharmaceutically important compounds by nonparametric rankings. *J. Pharm. Biomed. Anal.* **2015**, *115*, 183–191. [CrossRef]

10. Valko, K.; Nunhuck, S.; Bevan, C.; Abraham, M.H.; Reynolds, D.P. Fast Gradient HPLC Method to Determine Compounds Binding to Human Serum Albumin. Relationships with Octanol/Water and Immobilized Artificial Membrane Lipophilicity. *J. Pharm. Sci.* **2003**, *92*, 2236–2248. [CrossRef]

11. Valko, K. Lipophilicity and biomimetic properties measured by HPLC to support drug discovery. *J. Pharm. Biomed. Anal.* **2016**, *130*, 35–54. [CrossRef] [PubMed]

12. Ciura, K.; Dziomba, S.Z. Application of separation methods for In Vitro prediction of blood–brain barrier permeability—The state of the art. *J. Pharm. Biomed. Anal.* **2020**, *177*, 112891. [CrossRef] [PubMed]

13. Milošević, N.; Janjić, N.; Milić, N.; Milanović, M.; Popović, J.; Antonowić, D. Pharmacokinetics and Toxicity Predictors of New s-Triazines, Herbicide Candidates, in Correlation with Chromatographic Retention Constants. *J. Agr. Food Chem.* **2014**, *62*, 8579–8585. [CrossRef] [PubMed]

14. Tsopelas, F.; Giaginis, C.; Tsantili-Kakoulidou, A. Lipophilicity and biomimetic properties to support drug discovery. *Expert Opin. Drug Discov.* **2017**, *12*, 885–896. [CrossRef]

15. Russo, G.; Grumetto, L.; Szucs, R.; Barbato, F.; Lynen, F. Screening therapeutics according to their uptake across the blood-brain barrier: A high throughput method based on immobilized artificial membrane liquid chromatography-diode-array-detection coupled to electrospray-time-of-flight mass spectrometry. *Eur. J. Pharm. Biopharm.* **2018**, *127*, 72–84. [CrossRef]

16. Stergiopoulos, C.; Tsopelas, F.; Valko, K.; Ochsenkühn-Petropoulou, M. The use of biomimetic chromatography to predict acute aquatic toxicity of pharmaceutical compounds. *Toxicol. Environ. Chem.* **2021**. [CrossRef]

17. Escuder-Gilabert, L.; Molero-Monfort, M.; Villanueva-Camañas, R.M.; Sagrado, S.; Medina-Hernández, M.J. Potential of biopartitioning micellar chromatography as an In Vitro technique for predicting drug penetration across the blood-brain barrier. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2004**, *807*, 193–201. [CrossRef]

18. Riiz-Ángel, M.; Garcia-Álvarez-Coque, M.; Berthod, A. New Insights and Recent Developments in Micellar Liquid Chromatography. *Sep. Pur. Rev.* **2009**, *38*, 45–96. [CrossRef]

19. Rambla-Alegre, M. Basic Principles of MLC. *Chromatogr. Res. Int.* **2012**, *2012*, 898520. [CrossRef]

20. Kalyankar, T.M.; Kulkarni, P.D.; Wadher, S.J.; Pekamwar, S.S. Applications of Micellar Liquid Chromatography in Bioanalysis: A Review. *J. App. Pharm. Sci.* **2014**, *4*, 128–134. [CrossRef]

21. Tsopelas, F.; Danias, P.; Pappa, A.; Tsantili-Kakoulidou, A. Biopartitioning micellar chromatography under different conditions: Insight into the retention mechanism and the potential to model biological processes. *J. Chromatogr. A* **2020**, *1621*, 461027. [CrossRef] [PubMed]

22. Foley, J.P. Critical compilation of solute-micelle binding constants and related parameters from micellar liquid chromatographic measurements. *Anal. Chim. Acta* **1990**, *231*, 237–247. [CrossRef]

23. Janicka, M. Correlations between Chromatographic Parameters and Bioactivity Predictors of Potential Herbicides. *J. Chrom. Sci.* **2014**, *52*, 676–684. [CrossRef]

24. Leeson, P.D. Molecular inflation, attrition and the rule of five. *Adv. Drug Deliv. Rev.* **2016**, *101*, 22–33. [CrossRef] [PubMed]

25. Toutain, P.L.; Bousquet-Mélou, A. Volumes of distribution. *J. Vet. Pharmacol. Therap.* **2004**, *27*, 441–453. [CrossRef]

26. Huang, L.; Wells, M.C.; Zhao, Z. A Practical Perspective on the evaluation of small Molecule CNS Penetration in Drug Discovery. *Drug Metabol. Lett.* **2019**, *13*, 78–94. [CrossRef]

27. Norinder, U.; Haberlein, M. Computational approaches to the prediction of the blood brain distribution. *Adv. Drug Deliv. Rev.* **2002**, *54*, 291–313. [CrossRef]

28. Clark, D.E. In Silico prediction of blood-brain barrier permeation. *Drug Discov. Today* **2003**, *8*, 927–933. [CrossRef]

29. Geldenhuys, W.J.; Mohammad, A.S.; Adkins, C.E.; Lockman, P.R. Molecular determinants of blood–brain barrier permeation. *Ther. Deliv.* **2015**, *6*, 961–971. [CrossRef]

30. Prausnitz, M.R.; Langer, R. Transdermal drug delivery. *Nat. Biotechnol.* **2008**, *26*, 1261−1268. [CrossRef]

31. Chen, C.-P.; Chen, C.-C.; Huang, C.-W.; Chang, Y.-C. Evaluating Molecular Properties involved in Transport of Small Molecules in Stratum Corneum: A Quantitative Structure-Activity Relationship for Skin Permeability. *Molecules* **2018**, *23*, 911. [CrossRef] [PubMed]

32. Aggarwal, M.; Fisher, P.; Hŭser, A.; Kluxen, F.M.; Parr-Dobrzanski, R.; Soufi, M.; Strupp, C.; Wiemann, C.; Billington, R. Assessment of an extended dataset of in vitro human dermal absorption studies on pesticides to determine default values, opportunities for read-across and influence of dilution on absorption. *Reg. Tox. Pharmacol.* **2015**, *72*, 58–70. [CrossRef] [PubMed]

33. Kerns, E.; Di, L. Pharmaceutical profiling in drug discovery. *Drug Disc. Today* **2003**, *8*, 316–323. [CrossRef]
34. Platts, J.A.; Abraham, M.H.; Hersey, A.; Butina, D. Estimation of Molecular Linear Free Energy Relationship Descriptors. 4. Correlation and Prediction of Cell Permeation. *Pharm. Res.* **2000**, *17*, 1013–1018. [CrossRef]
35. Carter, D.C.; Ho, J.X. Strucyure of Serum Albumin. *Adv. Protein Chem.* **1994**, *45*, 153–176. [CrossRef]
36. Kratochwill, N.; Huber, W.; Muller, F.; Kansy, M.; Gerber, P.R. Predicting plasma protein binding of drugs-revisited. *Curr. Opin. Drug Discov. Dev.* **2004**, *4*, 507–512.
37. Hollósy, F.; Valkó, K.; Hersey, A.; Nunhuck, S.; Kéri, G.; Bevan, C. Estimation of Volume of Distribution in Humans from High Throughput HPLC-Based Measurements of Human Serum Albumin Binding and Immobilized Artificial Membrane Partitioning. *J. Med. Chem.* **2006**, *49*, 6958–6971. [CrossRef]
38. Baur, P.; Marzouk, H.; Schőnherr, J.; Grayson, B.T. Partition Coefficients of Active Ingredients between Plant Cuticle and Adjuvants as Related to Rates of Foliar Uptake. *J. Agric. Food. Chem.* **1997**, *45*, 3659–3665. [CrossRef]
39. Karlsson, J.O.G. Preclinical safety assessment of contrast media: Predictive value. *Eur. Radiol.* **1996**, *6*, S3–S7. [CrossRef]
40. Quiñones-Torrelo, C.; Sagrado-Vives, S.; Villanueva-Camañas, R.M.; Medina-Hernández, M.J. An LD50 model for predicting psychotropic drug toxicity using biopartitioning micellar chromatography. *Biomed. Chromatogr.* **2001**, *15*, 31–40. [CrossRef]
41. Wang, J.; Skolnik, S. Recent Advances in Physicochemical and ADMET Profiling in Drug Discovery. *Chem. Biodiver.* **2009**, *6*, 1887–1899. [CrossRef] [PubMed]
42. Rácz, A.; Bajusz, D.; Miranda-Quintana, R.A.; Héberger, K. Machine learning models for classification task related to drug safety. *Mol. Diver.* **2021**, *25*, 1409–1424. [CrossRef]
43. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3–26. [CrossRef]
44. Lipinski, C.A. Rule of five in 2015 and beyond: Target and ligand structural limitations, ligand chemistry structure and drug discovery project decisions. *Adv. Drug. Deliv. Rev.* **2016**, *101*, 34–41. [CrossRef] [PubMed]
45. Gramatica, P. On the development and Validation of QSAR Models. In *Computational Toxicology*; Reisfeld, B., Mayeno, A.N., Eds.; Springer: Berlin/Heidelberg, Germany; Humana Press: Totowa, NJ, USA, 2013; Volume II. [CrossRef]

ORIGINAL RESEARCH PAPER

Predicting pharmacokinetic properties of potential anti-cancer agents using micellar thin-layer chromatography

Małgorzata Janicka[1] ⬤ · Anna Śliwińska[2] · Małgorzata Sztanke[3] · Krzysztof Sztanke[4]

Abstract

The thin-layer chromatography technique with a micellar mobile phase was used to predict the pharmacokinetic properties of newly synthesized azaisocytosine-containing congeners that are considered as potential anti-cancer drugs. TLC RP-CN pre-coated plates were applied as the stationary phase, and buffered (pH = 7.4) 0.1 M sodium dodecyl sulphate with 20% (*V/V*) of tetrahydrofuran was used as the mobile phase. The quantitative structure−activity relationships (QSARs) methodology was used to predict the compounds' blood−brain distribution (log *BB*), the unbounded fraction in the brain ($f_{u,brain}$), and their binding to the human plasma proteins (log $K_{a,HSA}$). The independent variables were the chromatographic lipophilicity, polarizability, and molecular weight. The statistically significant, quantitative structure−activity relationships that were established highlighted the significance and possibilities of the planar technique in predicting the pharmacokinetic properties of organic compounds in screening investigations. The procedure for restoring precoated plates and re-using them in chromatography studies was presented. The results confirmed the advantages of the planar chromatography technique that makes it possible to use chromatographic plates multiple times without significant deterioration of the efficiency and repeatability of the retention data.

Keywords Micellar chromatography · Sandwich chambers · Lipophilicity · Quantitative structure−activity relationships (QSARs)

1 Introduction

Thin-layer chromatography (TLC) is an analytical technique with many advantages, including the low consumption of reagents, the possibility of conducting multiple analyses simultaneously, and the relatively low costs of the apparatus and a single analysis. However, the TLC technique has some limitations, mainly related to the single use of the chromatographic plates. The high cost of pre-coated plates is the reason this technique is losing its competitiveness over column chromatography. The aim of this work was to evaluate the possibility of using re-usable plates, especially in pilot studies, which would allow the optimization of the research costs and compliance with green chemistry assumptions [1]. In the studies, the planar chromatography technique with a micellar mobile phase was used to predict the important pharmacokinetic properties of newly synthesized twenty-one fused azaisocytosine-containing congeners (Table 1), which were being investigated for potential use as drugs [2, 3].

The biological activities of various compounds can be related to their lipophilic, electric, and steric properties, and they can be expressed as a multiple linear regression (MLR) [4–6]:

✉ Małgorzata Janicka
malgorzata.janicka@mail.umcs.pl
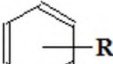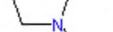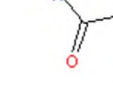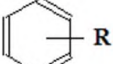
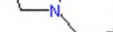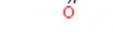1 Department of Physical Chemistry, Faculty of Chemistry, Institute of Chemical Science, Maria Curie-Skłodowska University, Lublin, Poland

2 Doctoral School of Quantitative and Natural Sciences, Maria Curie-Skłodowska University, Lublin, Poland

3 Chair and Department of Medical Chemistry, Medical University of Lublin, Lublin, Poland

4 Laboratory of Bioorganic Synthesis and Analysis, Chair and Department of Medical Chemistry, Medical University of Lublin, Lublin, Poland

Table 1 The compounds tested and their structures, molecular weights (*MW*), polarizabilities (*α*), pharmacokinetics (log $K_{a,HSA}$, log *BB*, Caco-2, $f_{u,brain}$), and chromatographic (*R*$_M$) parameters

No.	R	Structure	MW [g mol^{-1}]	α [Å^3]	log $K_{a,HSA}$	log BB	$f_{u,brain}$	R_M	$R_M^{average}$
1	H		256.30	29.30	4.80	0.230	0.52	0.42	0.48
2	4-CH$_3$		270.33	31.06	4.86	0.423	0.32	0.53	0.60
3	2-Cl		290.75	31.13	5.09	0.339	0.33	0.45	0.55
4	3-Cl		290.75	31.13	5.07	0.384	0.28	0.54	0.63
5	4-Cl		290.75	31.13	5.16	0.328	0.38	0.53	0.62
6	3,4-Cl$_2$		325.19	32.95	5.44	0.473	0.21	0.53	0.60
7	H		282.22	25.85	4.96	0.102	0.44	0.65	0.78
8	2-CH$_3$		296.25	27.61	5.01	0.290	0.27	0.65	0.77
9	4-CH$_3$		296.25	27.61	5.01	0.290	0.27	0.74	0.89
10	2-OCH$_3$		312.25	28.16	5.03	0.063	0.43	0.55	0.62
11	2-Cl		316.67	27.68	5.29	0.211	0.27	0.65	0.73
12	3-Cl		316.67	27.68	5.31	0.264	0.26	0.72	0.84
13	4-Cl		316.67	27.68	5.33	0.194	0.29	0.71	0.81
14	3,4-Cl$_2$		351.11	29.50	5.64	0.345	0.14	0.68	0.74

$$\text{Activity} = aA + bB + cC + \cdots + \text{const} = f(\text{lipophilic, electric, steric properties}) \tag{1}$$

where *a*, *b*, *c*, etc., are the correlation factors. The molecular descriptors (*A*, *B*, *C*, …) that relate to the lipophilic, electric, and steric properties of the molecule can be determined experimentally or evaluated in silico. Retention parameters, measured by column or planar reversed-phase liquid chromatography techniques, are the most popular, and they are accepted as chromatographic lipophilicity descriptors. In addition, different liquid chromatography techniques can be used as indirect in vitro methods for the determination of lipophilicity [7, 8]. These techniques include the novel types of columns that mimic the *n*-octanol–water system (*e.g.* polymeric reversed-phase columns), biological partitioning (immobilized artificial membranes or columns with immobilized cholesterol, human, or rat serum albumins, glycoproteins, and others [9–11]). Similar possibilities offer micellar liquid chromatography using surfactants as components of the mobile phase. The hydrophilic/hydrophobic nature of the micelles in the bulk phase and the surfactant in the modified stationary phase structurally resemble the ordered array of the hydrocarbon chains in the biomembranes [12–15].

The important biological properties of substances being considered as potential drugs are their ability to bind blood proteins and penetrate biological barriers, *e.g.* the blood–brain barrier. Human serum albumin (HSA) is one of the most abundant plasma proteins in the blood, and it has the important role of transporting drugs in a living organism.

The investigation of a drug's binding to HSA is required to understand the pharmacodynamics and pharmacokinetics profile of the drug [16]. The permeability of the blood–brain barrier must be known, and it should be assessed as early as possible in the testing stage, both for substances considered as potential central nervous system agents and peripherally acting drugs, in order to avoid harmful side effects [17–19]. In our research, the above-mentioned properties were described by the following parameters, *i.e.* the logarithm of the binding constant of albumin–substance interaction (log $K_{a,HSA}$), log *BB* (*BB* = brain concentration/blood concentration), and the unbonded fraction of the compound in the brain ($f_{u,brain}$).

2 Experimental

2.1 Chemicals

The chemicals used in the tests, *i.e.* acetonitrile (Chempur, Piekary Śląskie, Poland), tetrahydrofuran, citric acid, sodium phosphate dibasic (POCh, Gliwice, Poland), sodium dodecyl sulphate (Merck, Warsaw, Poland) were analytical grade. Deionized water was produced using the Direct-Q3 UV system (Millipore, Warsaw, Poland).

2.2 Test compounds

The compounds tested are two novel classes of fused azaisocytosine-containing congeners (Table 1). They had been synthesized in our laboratory and were described in our earlier work [2, 3]. Due to the expected anti-cancer activity, the substances require more thorough and extensive research.

2.3 Instrumentation and chromatographic conditions

TLC RP-CN (10×10 cm) pre-coated plates (Merck, Poland) were applied as stationary phases. Micellar mobile phase, *i.e.* buffered 0.1 M sodium dodecyl sulphate with 20% (*V/V*) of tetrahydrofuran, was used. The buffer (pH = 7.4) was prepared from 0.01 mol/L solutions of sodium phosphate dibasic and citric acid.

The substances dissolved in an amount of 1.0 mg in 1.0 mL of acetonitrile were applied on the plates automatically with the use of Linomat 4 (CAMAG, Warsaw, Poland). The plates were developed in a glass chromatographic sandwich chamber (Chromdes, Lublin, Poland) at room temperature. The spots were detected in ultraviolet (UV) light at 254 nm by means of a Reprostar 3 video camera and CAMAG VideoScan software (CAMAG, Poland).

2.4 The procedure of the restoration of TLC plates

The following plate restoration procedure was used. First, the surfactant was removed from the surface by immersing the plate in a chamber filled with distilled water for 24 h. The plate was dried at 60 °C (Plate Heater III, CAMAG, Poland) for about 30 min, and the dry and cool plate was immersed again for an additional 24 h in the same chamber filled with acetonitrile to remove the test substances that were applied. In the procedure, Stahl's chambers were used and the liquid volume was to ensure complete immersion of the chromatographic plate. No disturbing effects, such as stationary phase soaking off from the glass, were observed. Later, the plate was dried again and re-used in the same experiment. During the research, the substances were applied in the same mode, and the direction of development was always the same. The plate was used five times, *i.e.* one use when new followed by regeneration for four subsequent uses.

2.5 In silico calculations

Molecular weight (*MW*), polarizability (α) of the tested compounds as well as pharmacokinetic parameters characterizing their distribution between the blood and brain (log *BB*), fraction unbounded in brain ($f_{u,brain}$), and binding to human plasma proteins (log $K_{a,HSA}$) were evaluated from molecular structures of compounds by ACD/Percepta software (Łódź, Poland).

2.6 Statistics

Linear regression (LR), partial last square (PLS), and multiple linear regression (MLR) were done employing the statistical software Minitab 16 (Minitab Inc., State College, PA, USA).

3 Results and discussion

The retardation factors, R_F, were determined according to the formula [20]:

$$R_F = \frac{a}{b} \tag{2}$$

where a is the migration distance of a substance, and b is the migration distance of the solvent front, which, in this experiment, means the line of visible demixion. The R_F values were converted to R_M using the following equation:

$$R_M = \log\left(\frac{1}{R_F} - 1\right) \tag{3}$$

All of the data are presented graphically in Fig. 1, and the correlations between the experimental R_M values are summarized in Table 2.

The change in the value of R_M after the first washing was noticeable, but it became smaller with each repetition. The higher R_M values obtained on the re-used plates are a consequence of the permanent modification of the stationary phase during subsequent developments as well as regeneration processes. The only deviation from the trends was observed for substance No. 9. The highest R_M value was observed after the second washing out of this substance. Despite all of the differences, very good linear correlations were obtained between different R_M values (Table 2). From the experimental data measured on the regenerated plates (R_{M1}–R_{M4}), the mean values ($R_M^{average}$) were calculated (Table 1). The distance of the mobile phase is the smallest for a new plate, but it increases significantly after the first washing out after which it remains almost the same for each subsequent use (Table 3).

When the same regenerated plate is used repeatedly in the chromatographic analysis, it is necessary to determine how this affects efficiency. For this purpose, we examined the number of theoretical plates (*N*) and the height equivalent to theoretical plate (*H*) calculated according to the following equations [20]:
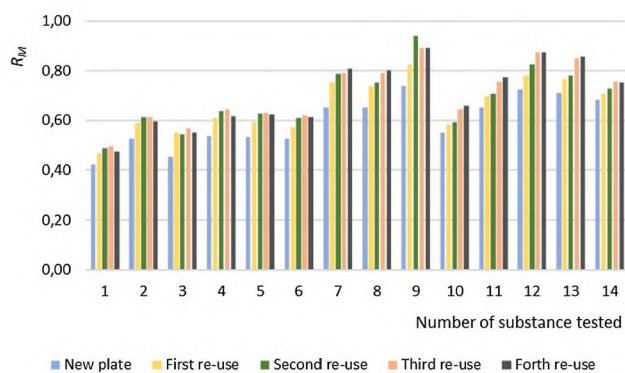
Fig. 1 Experimental R_M values

Table 2 Correlation matrix (values of correlation factor R) obtained for chromatographic parameters

Parameter	R_{M1}	R_{M2}	R_{M3}	R_{M4}
R_M	0.9734	0.9518	0.9849	0.9829
R_{M1}*	–	0.9783	0.9915	0.9869
R_{M2}	–	–	0.9701	0.9576
R_{M3}	–	–	–	0.9960

*Subscripts indicate the multiplicity of the plate restoration

**Table 3** Migration distance of the mobile phase for each experiment

Plate characteristics	Migration distance [mm]
New plate	75.99
First re-use	82.39
Second re-use	86.82
Third re-use	86.24
Forth re-use	85.94

$$N = \left(\frac{a}{\sigma_s}\right)^2 = 16\left(\frac{a}{w}\right)^2 \qquad (4)$$

where σ_s is the standard deviation, w ($w = 4\sigma_s$) is the peak width at the base, and:

$$H = \frac{b}{N} \qquad (5)$$

All of the data that were obtained are presented in Figs. 2 and 3, and the correlation between the number of theoretical plates and the height equivalent to a theoretical plate is shown in Fig. 3 as the N versus H relationship. In almost cases, the number of theoretical plates is the highest for a new plate. The main trend observed is a decrease in the
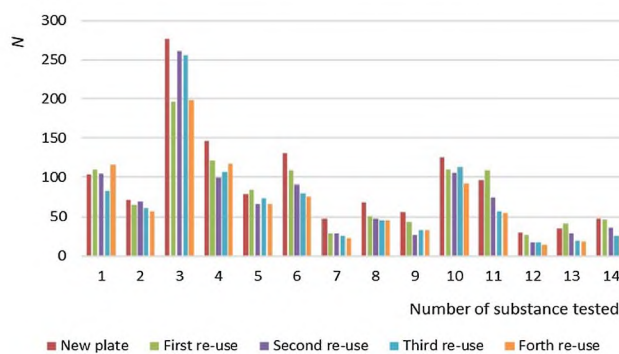
Fig. 2 The numbers of theoretical plates (N)
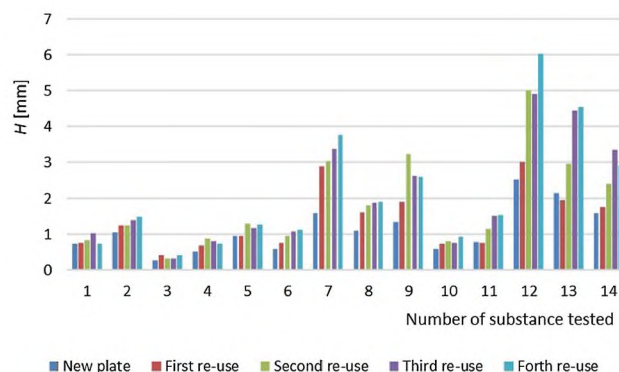
Fig. 3 The heights equivalent to theoretical plates (H)

number of theoretical plates (*i.e.* the increase in the height equivalent to a theoretical plate) with the multiple of chromatographic development. Of course, this tendency is analogous to the trend of changes for R_M values, and it confirms that the restoration procedure has an impact on the surface properties of the stationary phase. The changes result from the fact that the stationary phase is modified permanently by the mobile phase components, *i.e.* the time of development, the distance of the mobile phase, and the increase in the diffusion in the stationary phase. These factors result in broadening the band and increasing the value of H. This effect is most pronounced for the first re-use, and it diminishes for each of the following re-uses. The relationships between the efficiency values, N, and the heights equivalent of theoretical plate, H, presented in Fig. 3 are typical [20] and quite similar to each other.

3.1 Establishment of quantitative structure—activity relationships

In the QSARs methodology, we used the experimental R_M values measured on the new plates as lipophilicity descriptors, and we also used the mean values ($R_M^{average}$) of the parameters measured on the regenerated plates (Fig. 1,

Table 1). In addition, we used the molecular weights (*MWs*) and the electronic descriptors (polarizability, α) of the compounds we tested. Table 4 shows the relationships (Eqs. 6–11) that were established to predict the values of log $K_{a,HSA}$, log BB, and $f_{u,brain}$. The relationships were validated, and the results are presented in Table 4. The statistical parameters allowed us to evaluate the derived QSAR equations as being very good. There were no significant cross-correlations between the parameters that characterized the substances, *i.e.* the values of the variance inflation factor (*VIF*) were less than 5.

The diagrams presented in Figs. 5, 6 and 7 show the standardized coefficients of Eqs. (6)–(11). They explain both the direction and the strength of the impact of a given structural descriptor on the calculated biological parameter. The lipophilic properties of compounds increase their binding to human serum albumin and to the lipids contained in biological membranes. In the investigations, positive effects of the R_M ($R_M^{average}$) values were observed on the log $K_{a,HSA}$ and log BB values. The same effect involves the polarizability of molecules, which increases the strength of the van der Waals interactions between the solutes and the albumin or lipids molecules [21]. As shown in Figs. 4 and 5, α had a positive and even stronger effect on the log $K_{a,HSA}$ and log BB values than lipophilicity had. In the case of log BB, polarizability seems to be the dominant positive factor. Molecular weight (*MW*) has a strong positive effect on binding compounds to human serum albumin (log $K_{a,HSA}$), but reduces their concentration in the brain [19, 22]. For our substances, for the molar weights in the range of 351.11−266.30 g·mol^{-1}, we observed a slight negative impact of the molecular weight on the log BB values (Fig. 5). These properties of the molecule increase its affinity for proteins, and the lipids reduce the
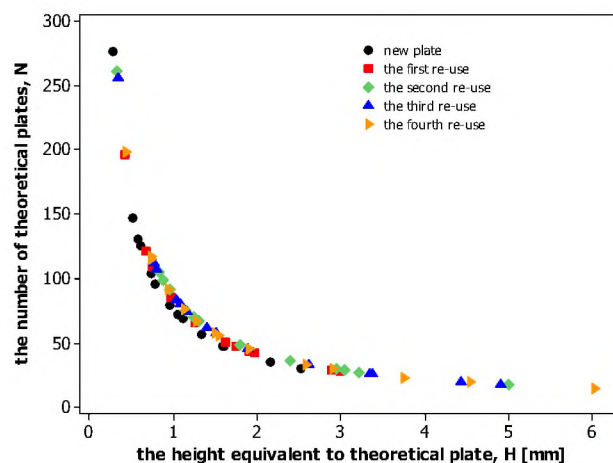

Fig. 4 The N versus H relationships

concentration of the free (unbonded) fraction in the brain. Figure 6 shows a strong negative impact of lipophilicity and polarizability and a slightly weaker impact of the molar weight on the values of $f_{u,brain}$.

The correlations shown in Figs. 5, 6 and 7 illustrate the relationships between the values calculated with the ACD/Percepta software (actual response) and those predicted by the QSARs models (calculated response) that were developed, *i.e.* from Eqs. (6)–(11). The applicability domain (AD) of the developed regression models was also evaluated and visualized as the Williams plots (Figs. 5, 6 and 7). AD is a theoretical region in physicochemical space (the response and chemical structure space) for which a QSAR model should make predictions with a given reliability [23]. The

Table 4 Established QSAR relationships

No. of Eq	QSAR relationships	n	R	R_{Adj}	sd	MSE	F	p	VIF
(6)	$\log K_{a,HSA} = 1.624(0.572) + 0.235(0.411)R_M$ $+ 0.028(0.017)\alpha + 0.009(0.001)MW$	14	0.9512	0.9361	0.082	0.007	32	0.0000	<3.5
(7)	$\log K_{a,HSA} = 1.647(0.595) + 0.148(0.316)R_M^{average}$ $+ 0.026(0.017)\alpha + 0.009(0.001)MW$	14	0.9507	0.9354	0.083	0.007	32	0.0000	<2.8
(8)	$\log BB = -2.018(0.325) + 0.944(0.234)R_M$ $+ 0.077(0.001)\alpha - 0.002(0.001)MW$	14	0.9347	0.9142	0.047	0.002	23	0.0001	<3.5
(9)	$\log BB = -2.059(0.352) + 0.700(0.187)R_M^{average}$ $+ 0.075(0.010)\alpha - 0.001(0.001)MW$	14	0.9281	0.9055	0.049	0.002	21	0.0001	<2.8
(10)	$f_{u,brain} = 2.339(0.304) - 0.847(0.219)R_M$ $- 0.039(0.009)\alpha - 0.001(0.001)MW$	14	0.9227	0.8982	0.044	0.002	20	0.0002	<3.5
(11)	$f_{u,brain} = 2.383(0.24) - 0.634(0.172)R_M^{average}$ $- 0.037(0.009)\alpha - 0.002(0.001)MW$	14	0.9178	0.8916	0.045	0.002	18	0.0002	<2.8

n—number of observations, R—correlation factor, $R_{Adj.}$—adjusted correlation factor, sd—standard deviation, F-value, p—probability value, VIF—variance inflation factor
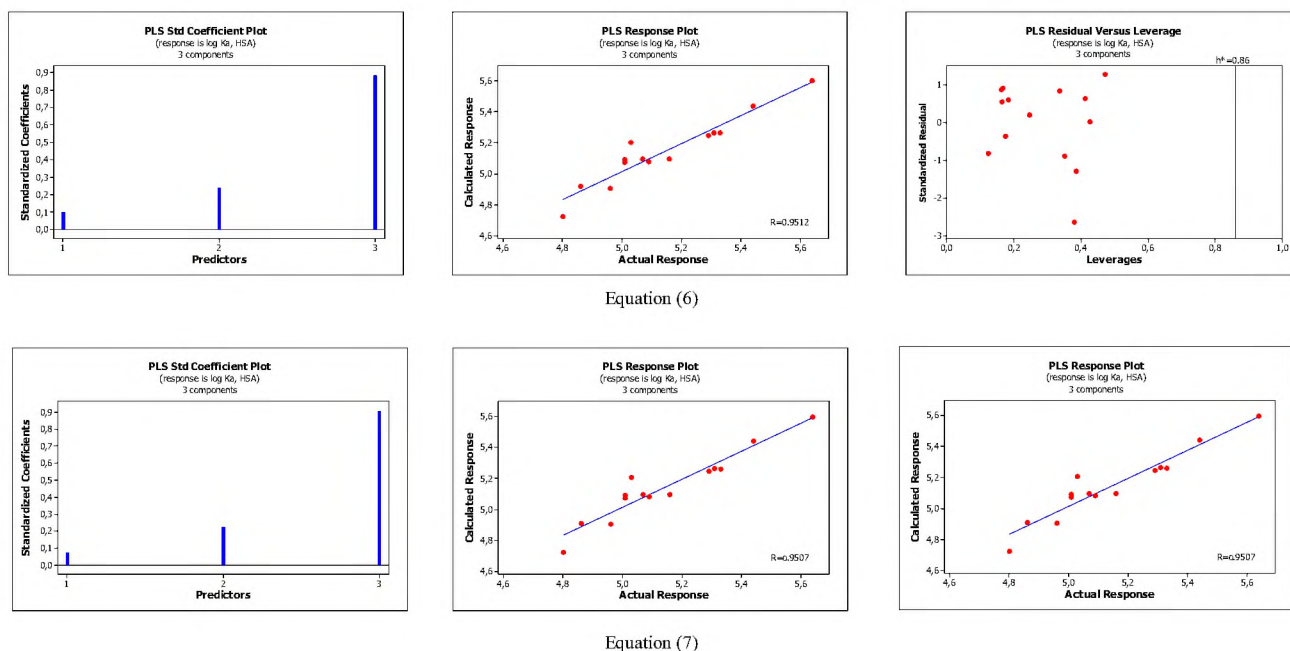
Fig. 5 Standardized coefficients, the correlation between actual and predicted values, and the Williams plots obtained for Eqs. (6) and (7)
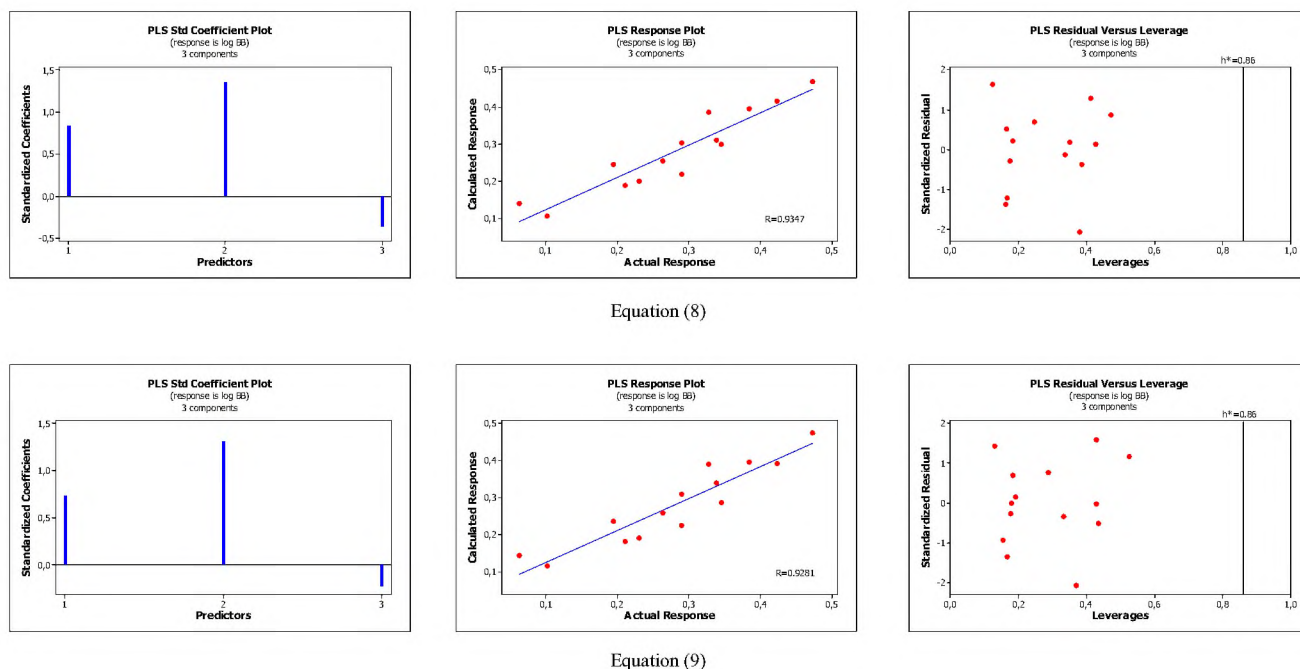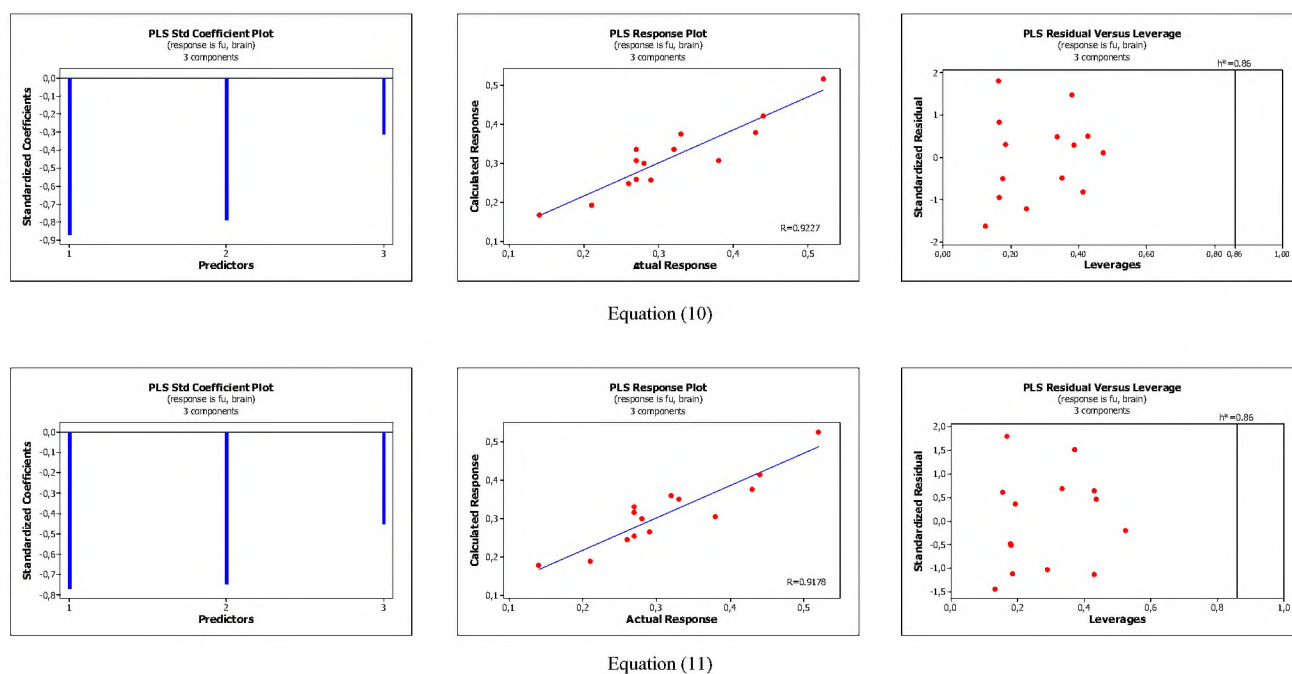

Fig. 6 Standardized coefficients, the correlation between actual and predicted values, and the Williams plots obtained for Eqs. (8) and (9)

following equation was used to calculate the warning leverage limits (h^*):

$$h^* = \frac{3(k+1)}{n} \tag{12}$$

where k is the number of descriptors used in the MLR model, and n is the number of compounds in the dataset. The Williams plot can be used for graphical detection of outliers ($h > h^*$) [23]. The results proved that the models that were obtained are valid within the domain in which they were developed.

Equation (10)

Equation (11)

Fig. 7 Standardized coefficients, the correlation between actual and predicted values, and the Williams plots obtained for Eqs. (10) and (11)

4 Conclusion

The two-dimensional QSAR methodology was successful in modelling pharmacokinetic properties—*i.e.* binding to human plasma proteins (log $K_{a,HSA}$), the distribution between the blood and the brain (log *BB*), and the unbounded fraction in the brain ($f_{u,brain}$) of new compounds being considered as potential drugs. The micellar chromatographic parameters measured using the TLC technique were proposed as lipophilicity descriptors, and, together with the molecular weight and polarizability, the descriptors were applied in the QSARs methodology. The equations that were derived were evaluated statistically and validated as being very good in that they provided a high predictive ability. The investigations highlighted the significance and advantages of planar chromatography resulting from the possibility of multiple re-use of chromatographic plates without significant deterioration of the efficiency and repeatability of retention data. The results that were obtained confirmed the importance of in vitro chromatographic techniques and QSARs methods in modelling the important pharmacokinetic properties of potential drugs in that they (1) reduce the extent of unethical animal testing, (2) reduce the time and costs of the screening experiments, and (3) follow the rules of green chemistry.

Declarations

Conflict of interest The authors declare no conflict of interest.

References

1. Anastas PT, Warner JC (1998) Green chemistry: theory and practice. Oxford University Press, New York, NY
2. Sztanke M, Rzymowska J, Janicka M, Sztanke K (2020) Two novel classes of fused azaisocytosine-containing congeners as promising drug candidates: design, synthesis as well as *in vitro, ex vivo* and *in silico* studies. Bioorg Chem 95:103480. https://doi.org/10.1016/j.bioorg.2019.103480
3. Janicka M, Mycka A, Sztanke M, Sztanke K (2021) Predicting pharmacokinetic properties of potential anticancer agents via their chromatographic behavior on different reversed phase materials. Int J Mol Sci 22:4257. https://doi.org/10.3390/ijms22084257
4. Hansch C (1993) Quantitative structure–activity relationships and the unnamed science. Acc Chem Res 26:147–153
5. Kaliszan R (1987) Quantitative structure–chromatographic retention relationships. John Wiley & Sons, New York, NY
6. Héberger K (2007) Quantitative structure–(chromatographic) retention relationships. J Chromatogr A 1158:273–305
7. Valkó K (2016) Lipophilicity and biomimetic properties measured by HPLC to support drug discovery. J Pharm Biomed Anal 130:35–54
8. Ciura K, Dziomba S (2020) Application of separation methods for *in vitro* prediction of blood–brain barrier permeability—The state of the art. J Pharm Biomed Anal 177:112891. https://doi.org/10.1016/j.jpba.2019.112891
9. Ayouni L, Cazarola G, Chaillou D, Herbreteau B, Rudaz S, Lantéri P, Carrupt P-A (2005) Fast determination of lipophilicity by HPLC. Chromatographia 62:251–255
10. Barbato FV, Cirocco L, Grumetto M, La Rotonda I (2007) Comparison between immobilized artificial membrane (IAM) HPLC data and lipophilicity in *n*-octanol for quinolone antibacterial agents. Eur J Pharm Sci 31:288–297
11. Kempińska D, Chmiel T, Kot-Wasik A, Mróz A, Mazerska Z, Namieśnik J (2019) State of the art and prospects of methods

for determination of lipophilicity of chemical compounds. Trends Anal Chem 113:54–73

12. Ruiz-Angel MJ, Carda-Broch S, Torres-Lapasió JR, García-Alvarez-Coque MC (2009) Retention mechanisms in micellar liquid chromatography. J Chromatogr A 1216:1798–1814

13. Tsopelas F, Panagiotis D, Pappa A, Tsantili-Kakoulidou A (2020) Biopartitioning micellar chromatography under different conditions: Insight into the retention mechanism and the potential to model biological processes. J Chromatogr A 1621:461027

14. Escuder-Gilabert L, Molero-Monfort M, Villanueva-Camaňas RM, Sagrado S, Medina-Hernández MJ (2004) Potential of biopartitioning micellar chromatography as an in vitro technique for predicting drug penetration across the blood–brain barrier. J Chromatogr B 807:193–201

15. Ciura K, Belka M, Kawczak P, Bączek T, Nowakowska J (2018) The comparative study of micellar TLC and RP-TLC as potential tools for lipophilicity assessment based on QSRR approach. J Pharm Biomed Anal 149:70–79. https://doi.org/10.1016/j.jpba.2017.10.034

16. Vuignier K, Schappler J, Veuthey J-L, Carrupt A (2010) Drug-protein binding: a critical review. Anal Bioanal Chem 398:53–66

17. Waterbeemd H, Camenisch G, Folkers G, Chretien JR, Raevsky OA (1998) Estimation of blood–brain barrier crossing of drugs using molecular size and shape, and H-bonding descriptors. J Drug Target 6:151–165

18. Feher M, Sourial E, Schmidt JM (2000) A simple model for the prediction of blood–brain partitioning. Int J Pharm 201:239–247

19. Geldenhuys WJ, Mohammad AS, Adkins CE, Lockman PR (2015) Molecular determinants of blood–brain barrier permeation. Ther Deliv 6:961–971. https://doi.org/10.4155/tde.15.32

20. Spangenberg B, Poole CF, Weins CH (2011) Quantitative thin-layer chromatography. A practical survey. Springer, Berlin

21. Tandon H, Ranjan R, Chakraborty T, Suhag V (2021) Polarizability: a promising descriptor to study chemical-biological interactions. Mol Divers 25:249–262

22. Hitchcok SA (2008) Blood-brain barrier permeability concentrations for CNS-targeted compound library design. Curr Opin Chem Biol 12:318–323

23. Gramatica P (2013) On the development and validation of QSAR Models. In: Reisfeld B, Mayeno AN (eds) Computational toxicology. Springer Nature, Berlin

Article

Combined Micellar Liquid Chromatography Technique and QSARs Modeling in Predicting the Blood–Brain Barrier Permeation of Heterocyclic Drug-like Compounds

Małgorzata Janicka [1,*], Anna Śliwińska [2], Małgorzata Sztanke [3] and Krzysztof Sztanke [4,*]

[1] Department of Physical Chemistry, Faculty of Chemistry, Institute of Chemical Science, Maria Curie-Skłodowska University, 20-031 Lublin, Poland
[2] Doctoral School of Quantitative and Natural Sciences, Maria Curie-Skłodowska University, 20-038 Lublin, Poland
[3] Department of Medical Chemistry, Medical University of Lublin, 4A Chodźki Street, 20-093 Lublin, Poland
[4] Laboratory of Bioorganic Compounds Synthesis and Analysis, Medical University of Lublin, 4A Chodźki Street, 20-093 Lublin, Poland
* Correspondence: malgorzata.janicka@mail.umcs.pl (M.J.); krzysztof.sztanke@umlub.pl (K.S.); Tel.: +48-814486195 (K.S.)

Abstract: The quantitative structure–activity relationship (QSAR) methodology was used to predict the blood–brain permeability (log *BB*) for 65 synthetic heterocyclic compounds tested as promising drug candidates. The compounds were characterized by different descriptors: lipophilicity, parachor, polarizability, molecular weight, number of hydrogen bond acceptors, number of rotatable bonds, and polar surface area. Lipophilic properties of the compounds were evaluated experimentally by micellar liquid chromatography (MLC). In the experiments, sodium dodecyl sulfate (SDS) as the effluent component and the ODS-2 column were used. Using multiple linear regression and leave-one-out cross-validation, we derived the statistically significant and highly predictive quantitative structure–activity relationship models. Thus, this study provides valuable information on the expected properties of the substances that can be used as a support tool in the design of new therapeutic agents.

Keywords: micellar chromatography; QSARs; log *BB*; lipophilicity; heterocyclic drug-like compounds

1. Introduction

The development of new drugs with desired properties is a tedious, laborious, time-consuming, and expensive process. Quantitative structure–activity relationship (QSAR) methods should be useful tools on this complicated path. The approach is based on the assumption that biological (pharmacokinetic) properties of structurally similar compounds can be quantitatively described by mathematical models. In addition, these models should predict with good probability the activity of structural analogs not yet synthesized. Although the establishment of QSAR models involves a number of steps and conditions, such as use of reliable and accurate input data, selection of relevant descriptors, and use of appropriate software and validation of the suggested model, the advantages of the QSAR methodology are not in doubt [1–8]. First of all, it reduces overhead costs, decreases the time of obtaining positive results, reduces animal testing, and respects the principles of Green Chemistry [5].

A crucial factor for a drug candidate is transport through the blood–brain barrier (BBB). Satisfactory transport through the BBB is an essential prerequisite for a potential drug to affect the central nervous system. However, to avoid side effects, the agents that act peripherally should not cross the BBB. In both cases, the permeability of the BBB must be known and should be evaluated at the earliest possible stage of testing [3–5,9–12].

The uptake of a compound into the brain is a complex process [13–16]. However, it is known that moderately lipophilic drugs can cross the BBB by passive diffusion [17–21]. A measure of the ability of a compound to penetrate the BBB is defined as the log *BB*, which is the ratio of the concentration of the drug molecules in the brain to the concentration in the blood at equilibrium [3]. The traditional method of measurement, requiring animal or human testing, is time-consuming, expensive, and complicated. For this reason, it is unacceptable in screening, and the indirect methods for log *BB* prediction are highly desired. QSAR methods combining in vitro and in silico techniques have become very attractive alternatives to in vivo testing [9]. Generally, QSAR models are functions of a molecule's structure, electronic properties, and lipophilicity. More or less sophisticated models using new descriptors selection have been proposed [22–32]. As polarity descriptors are used polar surface area (*PSA*) or topological polar surface area (*TPSA*) [33,34], the numbers of hydrogen bond donors (*HBD)* and hydrogen bond acceptors (*HBA)* [4,35,36]. Molecular size is characterized by molar weight, polarizability parameter, parachor, refractivity, molar volume, etc. [7,10]. Lipophilicity is usually evaluated using the log $P_{o/w}$ parameter describing solute partitioning between water and *n*-octanol.

For many years, various liquid chromatography techniques have been known and accepted to predict the lipophilic properties of organic substances, especially bioactive ones, as an alternative for the shake-flask technique. The theoretical bases are Collander-type equations [37], confirming good relationships between log $P_{o/w}$ (the logarithm of partition coefficient in the *n*-octanol/water system) and chromatographic parameters. In particular, the log k_w parameter corresponding to the retention of the solute in water as the mobile phase is considered as the lipophilicity descriptor [37–42].

Chromatographic techniques assessing the lipophilic properties of substances contribute to the development of QRAR (quantitative retention–property relationship) and/or QSAR (quantitative structure–activity relationship) models for predicting the penetration of substances through the BBB. There are different reversed-phase liquid chromatography techniques, both planar and column [37–45], used in this type of research. Chromatography with stationary phases that imitate biological partitioning systems, such as an artificial membrane, phases with immobilized lipids, albumin, cholesterol, ceramides, or liposomes, allow not only the prediction of the lipophilic properties [37–45] but also the behaviors of solutes in real biological systems (bounding to serum albumin, skin permeation, blood–brain barrier permeability, intestinal absorption, concentration of unbound form in blood, and others) [46–51].

Similar possibilities are offered by micellar liquid chromatography (MLC) using surfactants as components of the mobile phase. MLC is using a surfactant solution above the critical micellization concentration (*cmc*). Under these conditions, the micelles form the so-called micellar pseudophase in the bulk phase. The surrounding bulk water or aqueous–organic mixture contains surfactant monomers in a concentration approximately equal to the *cmc*. Moreover, surfactant monomers modify the surface phase as a result of the hydrophobic interactions between the tail of the surfactant and the alkyl chain grafted to the carrier surface of the stationary phase. Molecular interactions present in this system, i.e., solute association with the polar head of the surfactant, solute penetration into the micelle core, and solute interactions with adsorbed surfactant and alkyl chains, affect retention by three different equilibria, which are (1) the solute distribution between the micelle (micellar pseudophase) and the bulk phase, (2) the solute partition between the stationary phase modified by the surfactant and the bulk phase, and (3) the direct transfer of solute molecules between the surfactant-modified surface and the micelle [52–56].

The effect of the concentration of the surfactant in the effluent on the retention of the solute can be described by Foley's equation [57], where the following relationship exists between the retention parameter, *k*, and the concentration of the surfactant in the effluent:

$$\frac{1}{k} = \frac{1}{k_m} + \frac{K_{AM}}{k_m}[M] \tag{1}$$

where [*M*] is the total concentration of surfactant in the mobile phase minus the critical micellization concentration, *cmc*, K_{AM} is the constant that describes solute–micelle binding, and k_m is the solute retention parameter at zero micellar concentration, i.e., at surfactant monomer concentration equal to *cmc*. The K_{AM} and k_m parameters can be evaluated from the slope and intercept of experimental $1/k$ vs. [*M*] relationships. Equation (1) describes a linear dependence with decreasing retention as the micelle concentration increases. This equation is valid for aqueous solutions of surfactant or mobile phases with the same concentrations of the organic modifier. The micellar retention parameter, $\log k_m$, is considered analogous to the $\log k_w$ value evaluated in RPLC. Thus, this parameter is considered as a lipophilicity descriptor, and Equation (1) is a simple way to achieve the indirect determination of the lipophilic properties of compounds. It is postulated that retention in micellar chromatography depends on the hydrophobic (lipophilic), electronic, and steric features of the compounds in a similar way as many pharmacokinetic phenomena. Additional similarity is indicated by the fact that the phospholipids, cholesterol, fatty acids, and triglycerides that are present in the extracellular and intracellular fluids also form micelles with proteins.

All the compounds (**1–65**) [58–74] investigated in present studies have been designed and synthesized in our laboratory. Their molecular structures—confirmed by spectroscopic methods in previous studies [58–64,66–71]—are presented in Table 1. The purity and homogeneity of all the compound samples (**1–65**) have been proven [75]. These heterocyclic molecules may be of pharmaceutical importance due to their promising anticancer [58–68,70], analgesic [58,59,69,71], antiviral, and antihemolytic [65] activities as well as their medical applicability [61–63,67–69,73,74], high purity, and thermal stability [72]. Compounds belonging to the particular structurally related classes, e.g., I (**1–6**), III (**12–17**), IV (**18–28**), V (**34, 37–40**), and VII (**61–65**), have been recognized as anticancer agent candidates, especially in the treatment of human cancers of the cervix, breast (**1–6**, **12–17, 18–28,** and **61–65**) [60–64], lung, ovary (**1–6** and **61–65**) [60–62], pharynx, tongue (**1–6**) [65], and in the therapy of human multiple myeloma (**12–17, 34, 37–40**) [63,64,70]. Simultaneously, most of these molecules — as promising drug candidates — proved to be low in toxicity to normal cells, including erythrocytes [60–68,70]. Compounds **1–3** were found to be safer for the early life stages of zebrafish than an antiviral agent acyclovir, while molecules **3–6** were equally safe as this pharmaceutic [65]. Additionally, all these heterocycles (**1–6**) showed a protective effect on oxidatively stressed red blood cells, which was stronger or comparable to that of ascorbic acid. It has been shown that molecule **6** is antivirally active, too [65]. Furthermore, many compounds with low in vivo toxicity (**8–11, 32, 34, 39, 42, 48, 51,** and **53**) were found to be analgesic active, with three structures (**8, 42,** and **51**) showing the strongest antinociceptive activity in the acetic acid-induced writhing test in mice [58,59,69,71]. The first two analytical procedures for quantifying the pharmacologically relevant molecules (**15** and **39**) in solution and in serum samples have recently been developed and disclosed [73,74].

In our investigation, for the first time, the lipophilic properties of all the compounds (**1–65**) were experimentally determined by micellar liquid chromatography (MLC). The micellar lipophilicity parameters from MLC (as an experimental in vitro technique) and in silico data were combined with the aim of building the most satisfactory models for the prediction of the BBB permeation of 65 heterocyclic drug-like compounds. The linear quantitative structure–activity relationship models were produced using multiple linear regression on a database that consisted of different lipophilicity, polarity, electronic, and molecular size descriptors. The predictive ability of the developed models was validated by leave-one-out cross-validation (LOOcv).

Table 1. Heterocyclic molecules (**1–65**) belonging to the particular classes (I–VII).

Class	General Structure	No	R_1	R_2
I		1	R_1 = H	—
		2	R_1 = 4-CH_3	—
		3	R_1 = 2-Cl	—
		4	R_1 = 3-Cl	—
		5	R_1 = 4-Cl	—
		6	R_1 = 3,4-Cl_2	—
II		7	R_1 = H	—
		8	R_1 = 4-CH_3	—
		9	R_1 = 4-OCH_3	—
		10	R_1 = 4-OC_2H_5	—
		11	R_1 = 4-Cl	—
III		12	R_1 = H	—
		13	R_1 = 4-CH_3	—
		14	R_1 = 4-OCH_3	—
		15	R_1 = 3-Cl	—
		16	R_1 = 4-Cl	—
		17	R_1 = 3,4-Cl_2	—
IV		18	R_1 = H	—
		19	R_1 = 2-CH_3	—
		20	R_1 = 4-CH_3	—
		21	R_1 = 2,3-$(CH_3)_2$	—
		22	R_1 = 2-OCH_3	—
		23	R_1 = 4-OCH_3	—
		24	R_1 = 2-Cl	—
		25	R_1 = 3-Cl	—
		26	R_1 = 4-Cl	—
		27	R_1 = 3,4-Cl_2	—
		28	R_1 = 2,6-Cl_2	—
V		29	R_1 = H	—
		30	R_1 = 2-CH_3	—
		31	R_1 = 3-CH_3	—
		32	R_1 = 4-CH_3	—
		33	R_1 = 2-OCH_3	—
		34	R_1 = 4-OCH_3	—
		35	R_1 = 4-OC_2H_5	—
		36	R_1 = 2,3-$(CH_3)_2$	—
		37	R_1 = 2-Cl	—
		38	R_1 = 3-Cl	—
		39	R_1 = 4-Cl	—
		40	R_1 = 3,4-Cl_2	—

Table 1. *Cont.*

Class	General Structure	No	R_1	R_2
VI		41	R_1 = H	R_2 = H
		42	R_1 = H	R_2 = 2-Cl
		43	R_1 = H	R_2 = 3-Cl
		44	R_1 = H	R_2 = 4-Cl
		45	R_1 = 4-CH$_3$	R_2 = H
		46	R_1 = 4-CH$_3$	R_2 = 4-CH$_3$
		47	R_1 = 4-CH$_3$	R_2 = 3-CH$_3$
		48	R_1 = 4-CH$_3$	R_2 = 2-Cl
		49	R_1 = 4-CH$_3$	R_2 = 3-Cl
		50	R_1 = 4-CH$_3$	R_2 = 4-Cl
		51	R_1 = 4-OC$_2$H$_5$	R_2 = H
		52	R_1 = 4-OC$_2$H$_5$	R_2 = 4-CH$_3$
		53	R_1 = 4-OC$_2$H$_5$	R_2 = 2-Cl
		54	R_1 = 4-OC$_2$H$_5$	R_2 = 3-Cl
		55	R_1 = 4-OC$_2$H$_5$	R_2 = 4-Cl
		56	R_1 = 2-CH$_3$	R_2 = 2-Cl
		57	R_1 = 4-Cl	R_2 = H
		58	R_1 = 4-Cl	R_2 = 2-Cl
		59	R_1 = 4-Cl	R_2 = 3-Cl
		60	R_1 = 4-Cl	R_2 = 4-Cl
VII		61	R_1 = H	—
		62	R_1 = 4-CH$_3$	—
		63	R_1 = 2-Cl	—
		64	R_1 = 4-Cl	—
		65	R_1 = 3,4-Cl$_2$	—

2. Results

Chromatographic retention parameters (k) were calculated according to the following relationship:

$$k = \frac{t_R - t_M}{t_M} \quad (2)$$

where t_R and t_M are retention times for a given solute and an unretained compound, respectively.

To describe the effect of surfactant concentration on solutes retention, we applied the Foley equation and four effluents with different SDS concentrations: 0.1, 0.105, 0.11, and 0.12 mol L^{-1}. The obtained results are presented in Figure 1 as the $1/k$ vs. [M] relationships (Equation (1)) for five chosen compounds. The parameters of Equation (1) for all compounds tested, together with coefficients of determination R^2, are presented in Table 2.

Physico-chemical parameters characterizing the investigated compounds, i.e., the logarithm of the partition coefficient (log $P_{o/w}$) in the *n*-octanol/water system, the numbers of hydrogen bond donors (*HBD*), acceptors (*HBA*), and rotatable bonds (*NRB*), molecular weight (*MW*), topological polar surface area (*TPSA*), polarizability (α), and parachor (P), are presented in Table 3. Also included are the log *BB* parameters. All the values were evaluated in silico from molecular structures (ACD Percepta software). The log *BB** parameters from Table 3 were calculated in our previous studies [75], using the equation derived for 23 structurally similar compounds with known experimental log *BB* values. The relationship between both, log *BB* and log *BB**, parameters is linear and very good ($R^2 = 0.9010$).
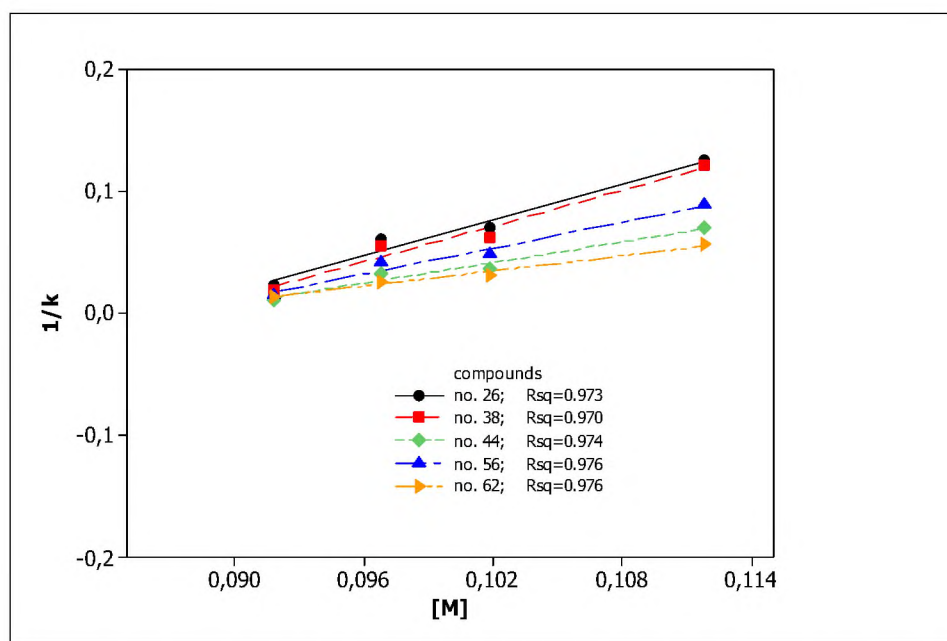
Figure 1. The $1/k$ vs. [M] relationships obtained for compounds **26**, **38**, **44**, **56**, and **62** from MLC measurements.

Table 2. Coefficients of determination and parameters of Equation (1) obtained for the particular compounds.

No	$1/k_m$	K_{AM}/k_m	R^2	No	$1/k_m$	K_{AM}/k_m	R^2
1	−0.765	9.066	0.9178	34	−0.329	3.829	0.9615
2	−0.586	6.826	0.9530	35	−0.321	3.685	0.9604
3	−0.758	8.907	0.9110	36	−0.432	5.006	0.9593
4	−0.528	6.157	0.9700	37	−0.425	4.933	0.9442
5	−0.518	6.028	0.9553	38	−0.425	4.870	0.9606
6	−0.406	4.722	0.9748	39	−0.288	3.326	0.9717
7	−1.046	12.573	0.9124	40	−0.214	2.470	0.9692
8	−0.785	9.294	0.9592	41	−0.354	4.119	0.9753
9	−1.163	13.801	0.9618	42	−0.289	3.322	0.9764
10	−0.876	10.274	0.9477	43	−0.225	2.944	0.9724
11	−0.707	8.316	0.9759	44	−0.246	2.822	0.9741
12	−0.760	8.902	0.9302	45	−0.257	2.976	0.9378
13	−0.568	6.593	0.9592	46	−0.206	2.361	0.9705
14	−0.565	7.520	0.9932	47	−0.190	2.198	0.9728
15	−0.509	5.898	0.9696	48	−0.199	2.272	0.9694
16	−0.503	5.825	0.9732	49	−0.213	2.448	0.9695
17	−0.395	4.569	0.9734	50	−0.174	2.001	0.9666
18	−0.599	7.079	0.9622	51	−0.295	3.399	0.9704
19	−0.597	6.995	0.9375	52	−0.211	2.441	0.9718
20	−0.410	4.836	0.9776	53	−0.227	2.606	0.9684
21	−0.594	6.814	0.9473	54	−0.285	3.202	0.9441
22	−0.458	5.274	0.9638	55	−0.191	2.203	0.9636
23	−0.474	5.694	0.9695	56	−0.309	3.549	0.9763
24	−1.131	12.985	0.9193	57	−0.540	6.358	0.9690
25	−0.692	7.917	0.9672	58	−0.180	2.073	0.9521
26	−0.420	4.868	0.9733	59	−0.185	2.118	0.9609
27	−0.639	7.247	0.9662	60	−0.157	1.820	0.9465
28	−0.461	5.307	0.9593	61	−0.241	2.856	0.9792
29	−0.382	4.464	0.9659	62	−0.175	2.051	0.9765
30	−0.451	5.916	0.9656	63	−0.193	2.343	0.9682
31	−0.301	3.477	0.9657	64	−0.151	1.802	0.9488
32	−0.287	3.322	0.9707	65	−0.132	1.550	0.9358
33	−0.479	5.531	0.9187				

Table 3. Characteristics of the tested compounds.

No	$\log (k_{\mathrm{m}}/K_{\mathrm{AM}})$	$\log BB$	$\log BB^*$ [75]	TPSA Å^2	HBD	HBA	NRB	MW g mol^{-1}	α Å^3	$\mathcal{P}$ m^3 mol^{-1}
1	−0.957	0.117	0.21	48.27	0	5	2	242.28	27.55	497.81
2	−0.834	0.305	0.32	48.27	0	5	2	256.30	29.30	528.90
3	−0.950	0.226	0.26	48.27	0	5	2	276.72	29.37	526.66
4	−0.789	0.270	0.30	48.27	0	5	2	276.72	29.37	526.66
5	−0.780	0.209	0.24	48.27	0	5	2	276.72	29.37	526.66
6	−0.674	0.360	0.34	48.27	0	5	2	311.17	31.20	555.51
7	−1.099	−0.243	−0.16	74.57	0	7	4	286.29	30.27	561.26
8	−0.968	−0.051	−0.04	74.57	0	7	4	300.31	32.02	592.35
9	−1.140	−0.293	−0.28	83.80	0	8	5	316.31	32.57	611.52
10	−1.012	−0.167	−0.20	83.80	0	8	6	330.34	34.40	650.13
11	−0.920	−0.151	−0.12	74.57	0	7	4	320.73	32.09	590.11
12	−0.949	−0.132	−0.10	74.57	0	7	5	300.31	32.09	604.35
13	−0.819	0.055	0.02	74.57	0	7	5	314.38	33.85	635.45
14	−0.876	−0.167	−0.20	83.80	0	8	6	330.34	34.40	599.87
15	−0.771	0.029	0.00	74.57	0	7	5	334.76	33.92	630.96
16	−0.766	−0.033	−0.05	74.57	0	7	5	334.76	33.92	650.13
17	−0.660	0.117	0.05	74.57	0	7	5	369.20	35.74	628.72
18	−0.850	0.038	0.06	61.41	0	6	2	280.31	30.82	628.72
19	−0.845	0.225	0.17	61.41	0	6	2	294.34	33.57	657.57
20	−0.684	0.225	0.17	61.41	0	6	2	294.34	32.57	578.01
21	−0.833	0.417	0.29	61.41	0	6	2	308.37	34.33	609.11
22	−0.722	0.006	−0.04	70.64	0	7	3	310.34	33.12	597.18
23	−0.755	0.003	−0.05	70.64	0	7	3	310.34	33.12	597.18
24	−1.113	0.141	0.11	61.41	0	6	2	314.75	32.64	575.77
25	−0.899	0.194	0.16	61.41	0	6	2	314.75	32.64	575.77
26	−0.687	0.130	0.09	61.41	0	6	2	314.75	32.64	575.77
27	−0.860	0.281	0.19	61.41	0	6	2	349.20	34.47	604.62
28	−0.725	0.297	0.21	61.41	0	6	2	349.20	34.47	604.62
29	−0.650	0.227	0.15	48.27	0	5	2	290.32	33.92	604.97
30	−0.772	0.407	0.26	48.27	0	5	2	304.35	35.68	636.07
31	−0.541	0.407	0.26	48.27	0	5	2	304.35	35.68	636.07
32	−0.521	0.407	0.26	48.27	0	5	2	304.35	35.68	636.07
33	−0.743	0.188	0.05	57.50	0	6	3	320.35	36.23	655.23
34	−0.583	0.172	0.04	57.50	0	6	3	320.35	36.23	655.23
35	−0.566	0.298	0.11	57.50	0	6	4	334.41	38.05	693.84
36	−0.699	0.594	0.38	48.27	0	5	2	318.37	37.43	667.16
37	−0.693	0.331	0.20	48.27	0	5	2	324.76	35.75	633.82
38	−0.682	0.376	0.25	48.27	0	5	2	324.76	35.75	633.82
39	−0.522	0.319	0.19	48.27	0	5	2	324.76	35.75	633.82
40	−0.393	0.465	0.29	48.27	0	5	2	359.21	37.57	662.67
41	−0.615	0.341	0.22	48.27	0	5	3	304.35	35.75	643.58
42	−0.521	0.459	0.28	48.27	0	5	3	338.82	37.57	672.43
43	−0.469	0.459	0.28	48.27	0	5	3	338.82	37.57	672.43
44	−0.451	0.459	0.28	48.27	0	5	3	338.82	37.57	672.43
45	−0.474	0.524	0.33	48.27	0	5	3	318.41	37.57	674.68
46	−0.373	0.712	0.45	48.27	0	5	3	332.44	39.26	705.77
47	−0.342	0.712	0.45	48.27	0	5	3	332.44	39.26	705.77
48	−0.356	0.635	0.38	48.27	0	5	3	352.85	39.26	703.53
49	−0.389	0.635	0.38	48.27	0	5	3	352.85	39.33	703.53
50	−0.302	0.635	0.38	48.27	0	5	3	352.85	39.33	703.53
51	−0.531	0.424	0.19	57.50	0	6	5	348.44	39.88	732.46
52	−0.387	0.602	0.37	57.50	0	6	5	362.47	41.63	763.55
53	−0.416	0.526	0.23	57.50	0	6	5	382.88	41.70	761.31
54	−0.505	0.526	0.23	57.50	0	6	5	382.88	41.70	761.31
55	−0.343	0.526	0.22	57.50	0	6	5	382.88	41.70	761.31
56	−0.550	0.635	0.38	48.27	0	5	3	352.85	39.33	703.53
57	−0.803	0.440	0.27	48.27	0	5	3	338.82	37.57	672.43

No	log (k_m/K_{AM})	log *BB*	log *BB** [75]	TPSA Å²	HBD	HBA	NRB	MW g mol⁻¹	α Å³	$\mathcal{P}$ m³ mol⁻¹
58	−0.317	0.551	0.31	48.27	0	5	3	373.27	39.40	701.28
59	−0.326	0.551	0.31	48.27	0	5	3	373.27	39.40	701.28
60	−0.260	0.551	0.31	48.27	0	5	3	373.27	39.40	701.28
61	−0.456	0.459	0.29	48.27	0	5	4	318.37	37.58	682.19
62	−0.312	0.651	0.41	48.27	0	5	4	332.40	39.33	713.29
63	−0.370	0.567	0.34	48.27	0	5	4	352.82	39.40	711.04
64	−0.256	0.551	0.33	48.27	0	5	4	352.82	39.40	711.04
65	−0.190	0.701	0.43	48.27	0	5	4	387.26	41.22	739.89

3. Discussion

3.1. Chromatographic Data

In our previous studies [75], all the compounds presented in Table 1 were analyzed using RP HPLC with three different stationary phases imitating biological partitioning: ODS (octadecylsilyl), IAM (artificial immobilized membrane), and Cholester (immobilized cholesterol). As lipophilicity descriptors, log k_w parameters were used, describing solute retention in the system with 100% aqueous mobile phase, calculated by linear extrapolation. Presently, to mimic biodistribution, we have used the micellar chromatography technique with SDS as the mobile phase component and the Foley equation to describe solute retention. Very good linear relationships ($R^2 > 0.9$) were obtained for all tested compounds (Figure 1, Table 2), confirming that the Foley equation correctly describes the retention of solutes in the tested chromatographic systems. Unfortunately, due to the strong retention of the tested compounds, the intercepts for all equations are negative. This is inconsistent with the physico-chemical interpretation of the regression coefficient of this equation: the intercept is equal to the reciprocal of k_m, i.e., the retention factor in the system in which the concentration of unbound surfactant ([M]) in the effluent is equal to zero. This value may in no case be less than zero. For this reason, we decided to use log (k_m/K_{AM}) values calculated from the slopes of Equation (1) as micellar lipophilicity descriptors of the compounds. The rationale is that both parameters (k_m and K_{AM}) characterize lipophilic properties of solutes: their affinity to the stationary phase modified by the surfactant (k_m) and binding to the micelles (K_{AM}). Moreover, in our previous research [76] on the group of pesticides, we compared both micellar parameters (log k_m and log K_{AM}), obtaining a very good rectilinear relationship with $R^2 = 0.9724$. To assess the correctness of our deductive reasoning, we examined the correlation between log (k_m/K_{AM}) values and partition coefficients log $P_{o/w}$ obtained in silico from molecular structures of compounds, commonly accepted as lipophilicity descriptors. They were compared with analogous relationships for other chromatographic lipophilicities (log k_w) evaluated for ODS, IAM, and Cholester columns. The graphs presented in Figure 2 show the correct (direct proportion) relationships with moderate fit ($R^2 > 0.6$) but the best one for the micellar parameter ($R^2 = 0.7980$). The correlations between different chromatographic parameters considered as lipophilicity descriptors (log k_w and log (k_m/K_{AM}) are also moderate—R^2 in the range 0.6002–0.6990. The above relationships confirm that micellar parameters can be considered as lipophilicity descriptors.

3.2. In Silico Data

The compounds investigated have in silico log *BB* values in the range −0.293–0.712 (Table 3), and they penetrate the blood–brain barrier better (log *BB* > 0) or weaker (log *BB* < 0). Without more in-depth research, it is impossible to decide which may be CNS-active. It is clear that CNS activity requires BBB permeation, but some drugs that are not CNS-active may still pass through the BBB and show no activity because they do not interact with any CNS targets. Similarly, some drugs with an expected peripheral site of action may pass through the BBB, leading to undesirable side effects on the CNS. Molar weights of

compounds range from 242.28 to 387.26 g mol^{-1} (Table 3) and meet the rule formulated by Lipiński and coworkers (The Rule of 5, Ro5) [77], i.e., $MW \leq 500$ g mol^{-1} or one of the "Rules of Thumb" proposed by Clark [78] ($MW \leq 450$ g mol^{-1}) for brain permeation by drugs and clinical candidates. The numbers of hydrogen bond acceptors $HBA \leq 10$, and the numbers of hydrogen bond donors $HBD \leq 5$. HBDs also fulfill Ro5. The investigated molecules are bases: $HBD = 0$ for all compounds [75], with HBA ranging from five to eight (Table 2). Moreover, the polar surface areas, described by $TPSA$ values, are in the range 48.27–57.50 Å^2, which meets the next principle given by Clark [78]. For good brain permeation, the polar surface area of the compound should be below a certain limit. In the literature on the subject, there are two differing limits: 90 Å^2 suggested by van de Waterbeemd et al. [36] and a lower limit of 60–70 Å^2 proposed by Kelder et al. [33]. The test substances (except compounds from groups II and III) satisfy both limits. Table 3 also provides values of parachor (P) ranging from 497.81 to 763.55 m^3 mol^{-1} and polarizability (α) ranging from 27.55 to 41.70 Å^3.
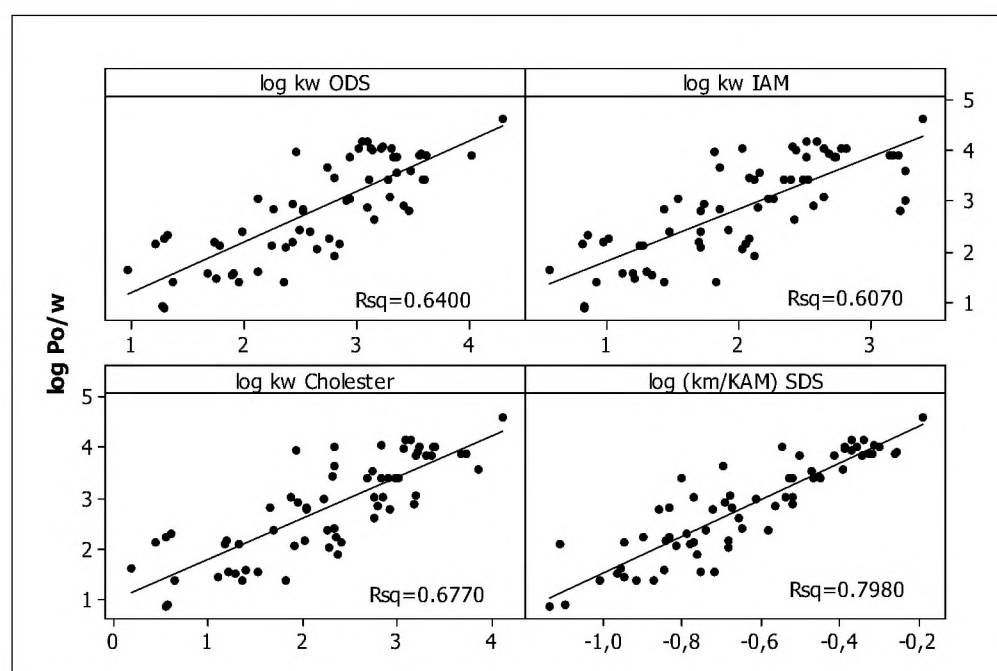
Figure 2. The relationships between log $P_{o/w}$ and log $k_{w,\,ODS}$, log $k_{w,\,IAM}$, log $k_{w,\,Cholester}$ [75], and log (k_m/K_{AM}) values.

In our procedure, parameters characterizing the lipophilic, structural, and electronic properties of molecules, i.e., micellar parameter (log k_m/K_{AM}), MW, $TPSA$, HBA, α, and P, will be used as independent variables. To ensure the variables have a minimal impact on each other (to keep the principle of orthogonality), we checked for similarities among them. The results are presented in Figure 3. Here, we can see three groups of strongly correlated descriptors: (I) including $TPSA$ and HBA (99.62%) characterizing the polar nature of the molecule and its ability to form hydrogen bonds; (II) consisting of polarizability, parachor, and molar weight (93.16%) related to the size of the molecule; and a single-element group (III) containing NRB describing molecule flexibility.

3.3. Establishment of Quantitative Structure–Activity Relationships

The establishment of QSAR models involves the use of reliable and accurate input data, selection of relevant descriptors, use of appropriate software, and validation of the suggested model [79,80]. Our models have involved descriptors characterizing solutes' lipophilicity (micellar log (k_m/K_{AM}) values), polarity ($TPSA$, HBA), flexibility (NRB), and size (MW, α, P). We used in silico (HBA, NRB, MW, $TPSA$, α, P, log BB, and log BB^*)

and in vitro ($\log k_m / K_{AM}$) data. The models were produced using the multiple linear regression (MLR) technique on a database that consisted of 65 recently discovered drug-like compounds. The linear quantitative structure–activity relationships (QSARs) were presented for the modeling of $\log BB$ values. The developed models were validated by leave-one-out cross-validation (LOOcv).
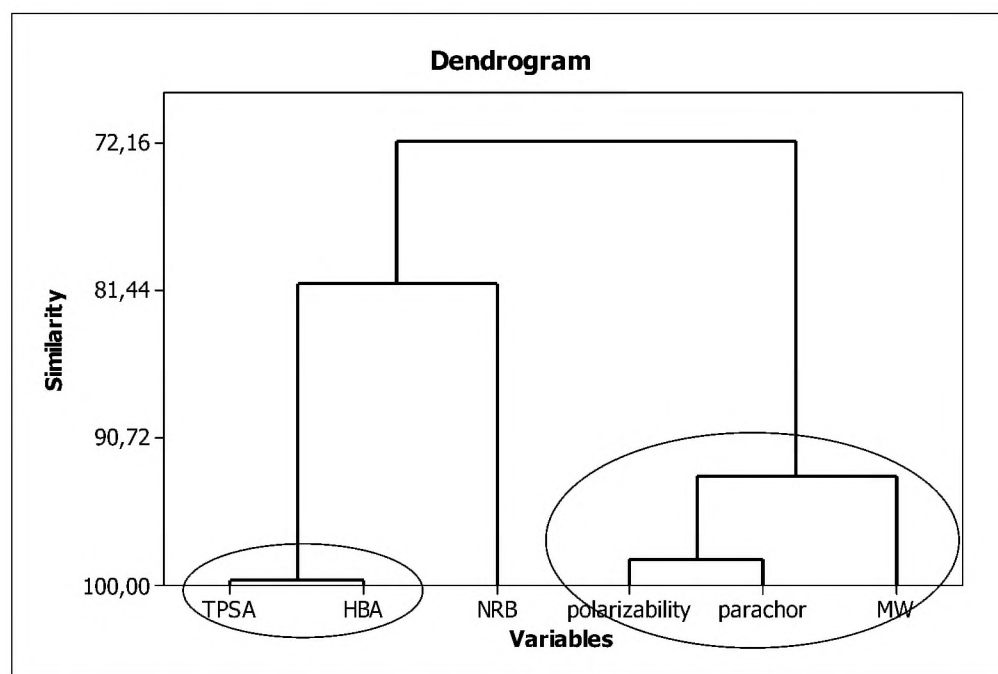

Figure 3. Similarities between the in silico molecular descriptors.

Validation is a necessary step to establish the quality of a QSAR model [81–83]. In our investigations, traditional validation metrics were applied: the mean squared error (*MSE*), the coefficient of determination (R^2), the determination coefficient adjusted (R^2_{adj}), and the determination coefficient predicted (R^2_{pred}). R^2_{adj} is used to compare the goodness-of-fit for regression models that contain differing numbers of independent variables while R^2_{pred} determines how well a regression model makes predictions. These coefficients (R^2, R^2_{adj}, R^2_{pred}) have values between zero and one, and the closer to one, the more accurate the model. The *MSE* is used to assess the predictive ability and accuracy of the model, and models with small *MSE* values yield more highly reliable predictions. The derived models were compared and assessed by leave-one-out cross-validation (LOO), and the resulting determination coefficient (Q^2LOO) and *PRESS* were calculated (Table 4). *PRESS* is a good estimate of the real prediction error of the model. It assesses a model's predictive ability and, in general, the smaller the *PRESS* value, the better the model's predictive ability [84]. The calculated global *PRESS* value must be lower than the sum of the squares of the response values of the total observations (*SS*). This proves that the developed models predict better than chance [83]. A reasonable QSAR model should have Q^2LOO values greater than 0.6 or the ratio of *PRESS/SS* smaller than 0.4 [84]. QSAR models are only valid in the domain they were validated [85] so the determination of applicability domain (AD) is of great importance [86]. AD is a space of (physico-chemical) information, on which the model has been developed and for which it is applicable to make predictions for new compounds. In the present work, we used the leverage approach (Williams plot) where the warning leverage, $h*$, was calculated according to:

$$h* = \frac{3(p+1)}{n} \tag{3}$$

where n is the total number of samples in, and p is the number of descriptors involved in the correlation [87].

Table 4. Statistics of the established models M1-M12, M5*, M11*, and M12*: the coefficient of determination (R^2, Q^2), the determination coefficient adjusted (R^2_{adj}), the determination coefficient predicted (R^2_{pred}), the predicted residual error sum of squares (*PRESS*), the variance inflation factor (*VIF*), the sum of squared differences from the mean (*SS*), the mean squared error (*MSE*), *F*-value, *p*-value; * the highest value.

Model	R^2	R^2_{adj}	R^2_{pred}	*PRESS*	*VIF**	*SS*	*MSE*	*F*	*p*	Q^2LOO	PRESS$_{LOO}$
M1: log *BB* vs. (log (k_m/K_{AM}), *TPSA*, *NRB*, α)	0.9202	0.9149	0.9088	0.3889	5.3	4.2636	0.0057	173.0	0.00000	–	–
M2: log *BB* vs. (log (k_m/K_{AM}), *TPSA*, *NRB*, $\mathcal{P}$)	0.9070	0.9008	0.8931	0.4556	4.7	4.2636	0.0066	146.3	0.00000	–	–
M3: log *BB* vs. (log (k_m/K_{AM}), *TPSA*, *NRB*, *MW*)	0.8943	0.8873	0.8799	0.5119	5.0	4.2636	0.0075	129.9	0.00000	–	–
M4: log *BB* vs. (log (k_m/K_{AM}), *HBA*, *NRB*, α)	0.9260	0.9210	0.9150	0.3625	5.4	4.2636	0.2774	187.6	0.00000	–	–
M5: log *BB* vs. (log (k_m/K_{AM}), *HBA*, *NRB*, $\mathcal{P}$)	0.9109	0.9049	0.8971	0.4385	4.7	4.2636	0.0063	153.3	0.00000	0.9109	0.4385
M6: log *BB* vs. (log (k_m/K_{AM}), *HBA*, *NRB*, *MW*)	0.8914	0.8841	0.8764	0.5268	4.8	4.2636	0.0077	123.1	0.00000	–	–
M7: log *BB** vs. (log (k_m/K_{AM}), *TPSA*, *NRB*, α)	0.8523	0.8425	0.8310	0.3247	5.3	1.9212	0.0047	86.6	0.00000	–	–
M8: log *BB** vs. (log (k_m/K_{AM}), *TPSA*, *NRB*, $\mathcal{P}$)	0.8508	0.8409	0.8297	0.3271	4.7	1.9212	0.0048	85.5	0.00000	–	–
M9: log *BB** vs. (log (k_m/K_{AM}), *TPSA*, *NRB*, *MW*)	0.8529	0.8430	0.8324	0.3219	5.0	1.9212	0.0047	86.9	0.00000	–	–
M10: log *BB** vs. (log (k_m/K_{AM}), *HBA*, *NRB*, α)	0.8682	0.8595	0.8489	0.2904	5.4	1.9212	0.0042	98.8	0.00000	–	–
M11: log *BB** vs. (log (k_m/K_{AM}), *HBA*, *NRB*, $\mathcal{P}$)	0.8659	0.8569	0.8466	0.2941	4.7	1.9212	0.0043	96.8	0.00000	0.8659	0.2947
M12: log *BB** vs. (log (k_m/K_{AM}), *HBA*, *NRB*, *MW*)	0.8662	0.8572	0.8473	0.2933	4.8	1.9211	0.0043	97.1	0.00000	0.8662	0.2933
M5*: log *BB* vs. (log (k_m/K_{AM}), *HBA*, $\mathcal{P}$)	0.9087	0.9042	0.8994	0.4290	4.7	4.2636	0.2178	202.4	0.00000	0.9087	0.4290
M11*: log *BB** vs. (log (k_m/K_{AM}), *HBA*, $\mathcal{P}$)	0.8656	0.8590	0.8513	0.2858	4.7	1.9212	0.0042	131.0	0.00000	0.8656	0.2858
M12*: log *BB** vs. (log (k_m/K_{AM}), *HBA*, *MW*)	0.8661	0.8595	0.8516	0.2852	4.1	1.9212	0.0042	131.5	0.00000	0.8515	0.2852

We searched for relationships between solute property (SP), i.e., log *BB*, and its lipophilicity, molar size, and flexibility descriptors, that is:

$$\log SP = a_0 + a_1 \text{lipophilicity} + a_2 d_I + a_3 d_{II} + a_4 d_{III} \tag{4}$$

where a_0–a_4 are regression coefficients, and d_I, d_{II}, and d_{III} are molecular descriptors from group I, II, and III, respectively.

Accounting for possible combinations of independent variables (descriptors from groups I, II, and III) for both parameters (log *BB* and log *BB**), twelve models denoted as M1-M6 (for log *BB*) and M7-M12 (for log *BB**) have been obtained. Table 4 contains the R^2, R^2_{adj}, R^2_{pred}, *PRESS*, and *VIF* (variance inflation factors) values calculated for these models, which became the basis for their preliminary evaluation leading to the selection of the most promising ones. Very high R^2 values (>>0.8) indicate that all M1-M12 equations are very good for modeling the data included (good feet of dataset). All R^2_{pred} values are >>0.6, indicating high predictive ability of the models. The decrease in the value of R^2_{adj} compared to the values of R^2 ranges from 0.0053 to 0.0099 units. High predictive abilities of the models are also confirmed by small *PRESS* values, which are in the range 0.2904−0.5268. Moreover, the ratios of *PRESS/SS* are smaller than 0.4. Variance inflation factors *VIF* should not exceed five to ensure that descriptors are moderately correlated. Some *VIF* values presented in Table 4 are equal to five or are slightly higher. For this reason, the M1, M3, M4, M7, M9, and M10 models were excluded from further analysis. Of the remaining six models, the most favorable were those with the highest R^2 and R^2_{pred} values and the lowest *PRESS*. The analysis of the calculated statistical parameters leads to the M5, M11, and M12 models as the most satisfactory. The models selected are as follows:

$$\text{M5: } \log BB = 0.253(0.232) + 0.198(0.091) \log (k_m/K_{AM}) - 0.160(0.023) \, HBA - 0.019(0.016) \, NRB + 0.002(0.000) \, P; \tag{5}$$

$$\text{M11: } \log BB^* = 0.842(0.191) + 0.106(0.075) \log (k_m/K_{AM}) - 0.148(0.019) \, HBA - 0.004(0.012) \, NRB + 0.0004(0.000) \, P; \tag{6}$$

$$\text{M12: } \log BB^* = 0.911(0.149) + 0.103(0.075) \log (k_m/K_{AM}) - 0.158(0.020) \, HBA + 0.002(0.011) \, NRB + 0.001(0.000) \, MW \tag{7}$$

Figures 4–6 present graphical results of LOO cross-validation of models M5, M11, and M12. The plots present in segments B illustrate the standard coefficients of the equations of selected models (Equations (5)–(7)). They explain both the direction and strength of the impact of a given descriptor on the calculated biological parameter. The correlations shown in segments A illustrate the relationships between the actual and predicted response, i.e., between log *BB* or log *BB** values from Table 3, and these predicted by the QSAR models were developed (Equations (5)–(7)). The applicability domain (AD) was also evaluated and visualized as the Williams plots (segments C). The results proved that the obtained models are valid within the domain for which they were developed.

The results (Figures 4B, 5B and 6B) indicate that the *NRB* parameter seems to be of negligible importance in the case of BBB permeation. Therefore, it was checked whether the omission of this descriptor would affect the evaluation of the derived models. The rationale is to capture the most important properties of compounds and build them into the QSAR model without overfitting the data. The results are presented as the following QSAR equations:

$$\text{M5*: } \log BB = 0.406(0.196) + 0.181(0.091) \log (k_m/K_{AM}) - 0.180(0.016) \, HBA + 0.002(0.000) \, P; \tag{8}$$

$$\text{M11*: } \log BB^* = 0.874(0.159) + 0.102(0.074) \log (k_m/K_{AM}) - 0.152(0.013) \, HBA + 0.0004(0.000) \, P; \tag{9}$$

$$\text{M12*: } \log BB^* = 0.903(0.139) + 0.108(0.069) \log (k_m/K_{AM}) - 0.156(0.013) \, HBA + 0.001(0.000) \, MW \tag{10}$$

The analysis of statistics of the above models, i.e., lower R^2_{adj}, R^2_{pred}, and *PRESS* values (Table 4), indicates that the new models (omitting the *NRB*) are better for predicting

substance permeation through the BBB. The new models were also cross-validated, and the results are presented graphically in Figures 7–9.
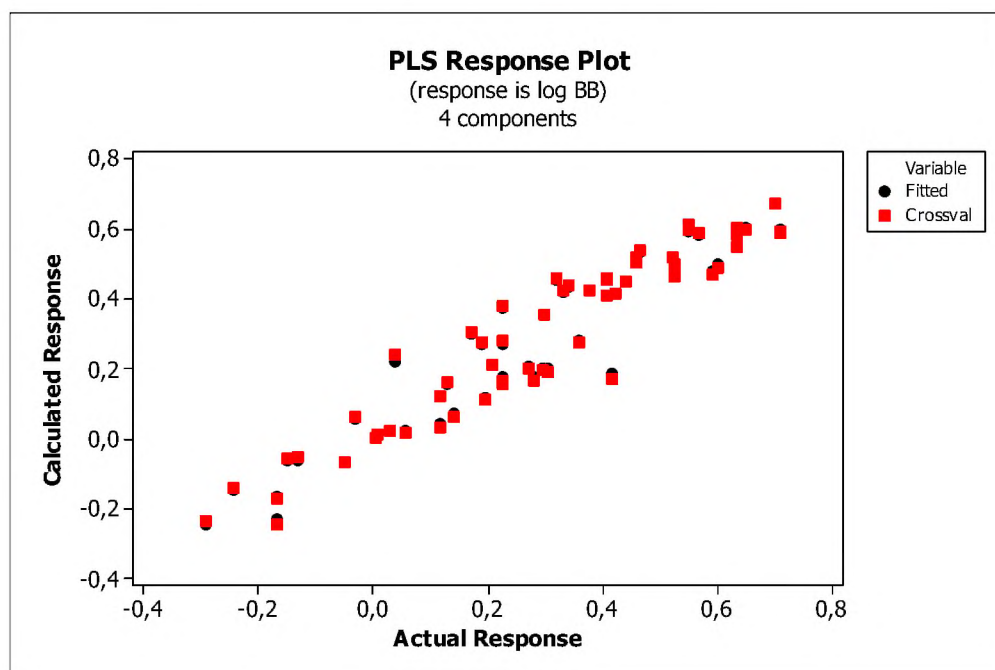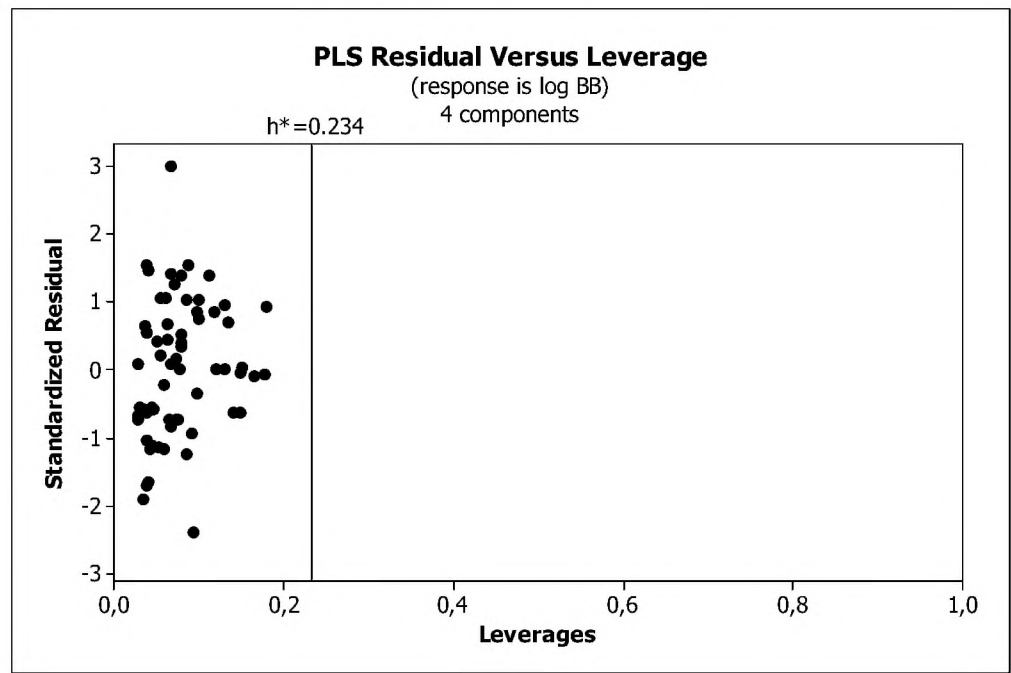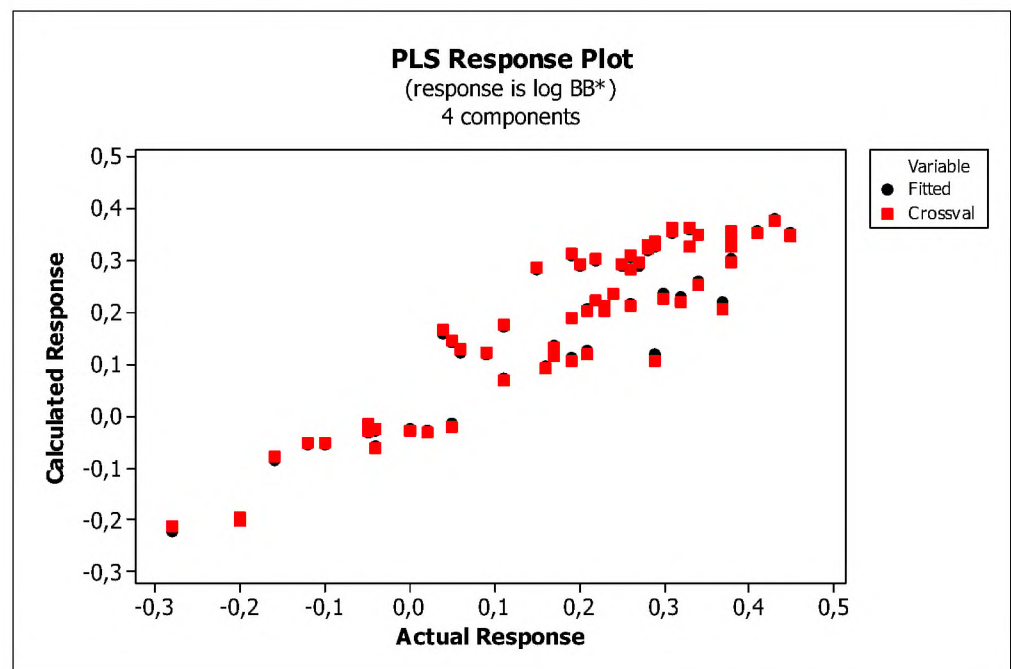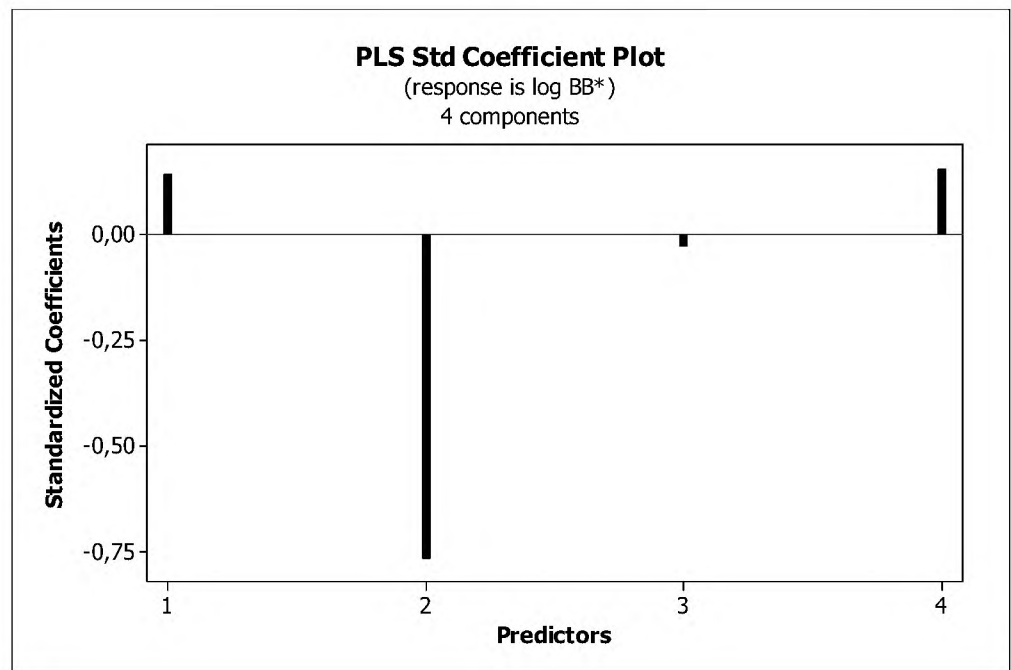

(A)
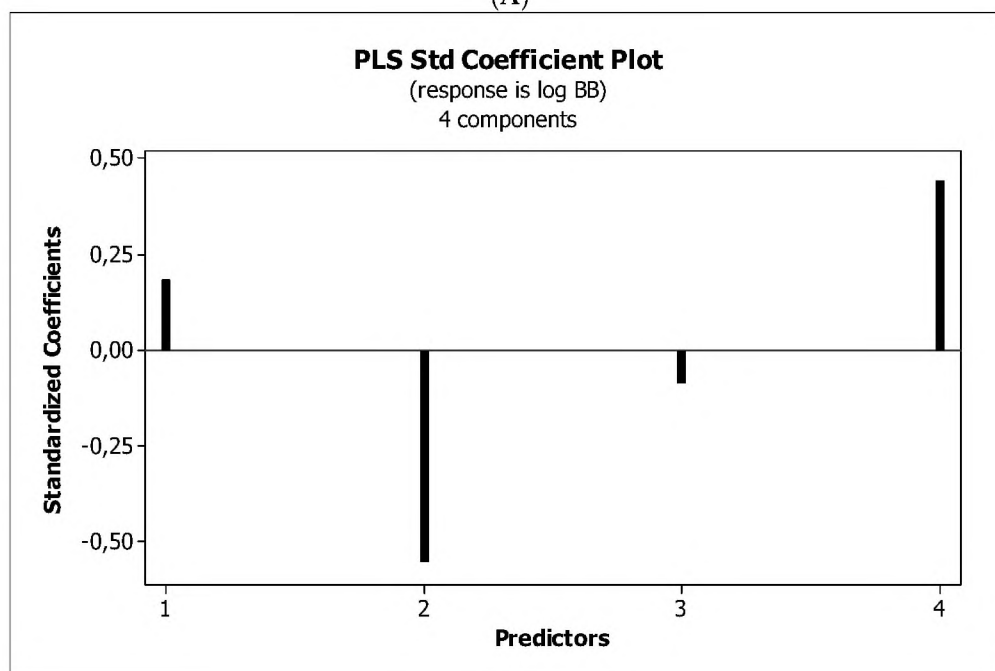
(B)

Figure 4. *Cont.*

(C)

Figure 4. (**A**) Model M5: the response plot. (**B**) Model M5: the standardized coefficients plot. (**C**) Model M5: Williams plot.
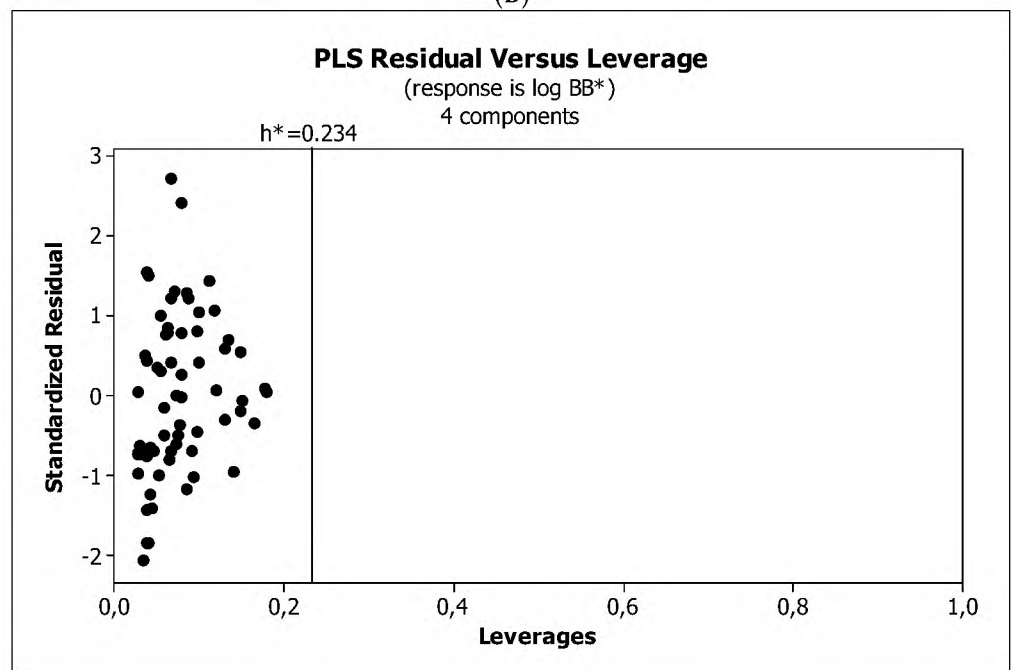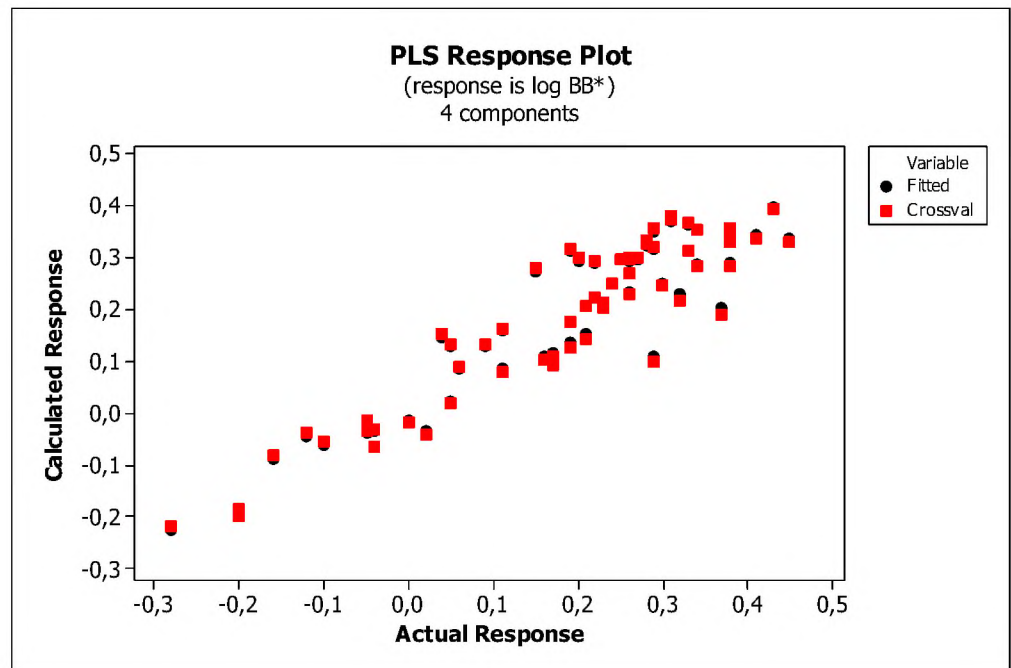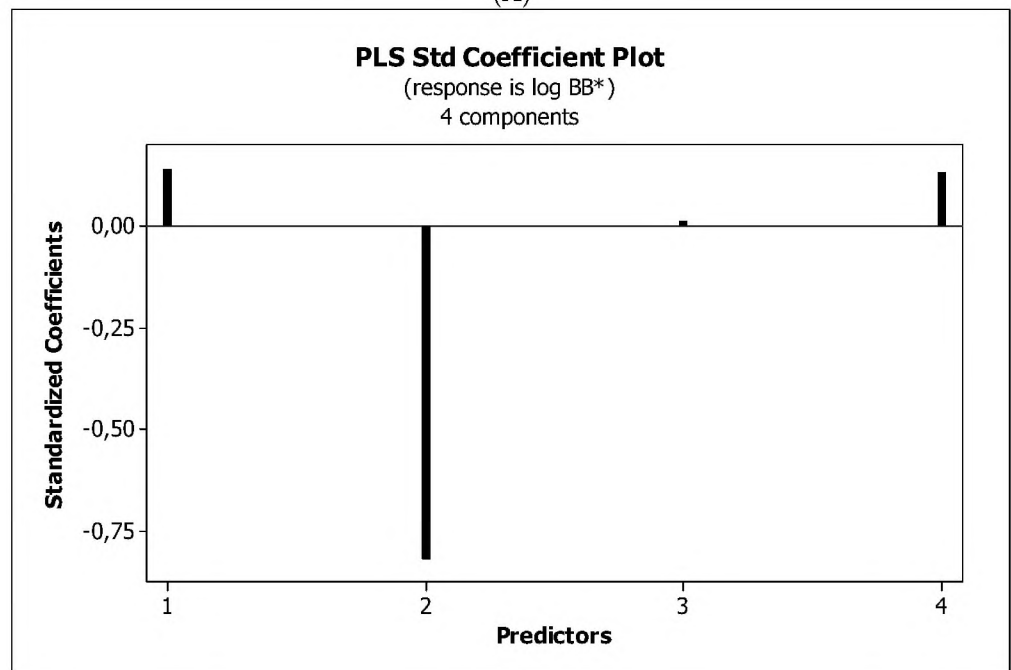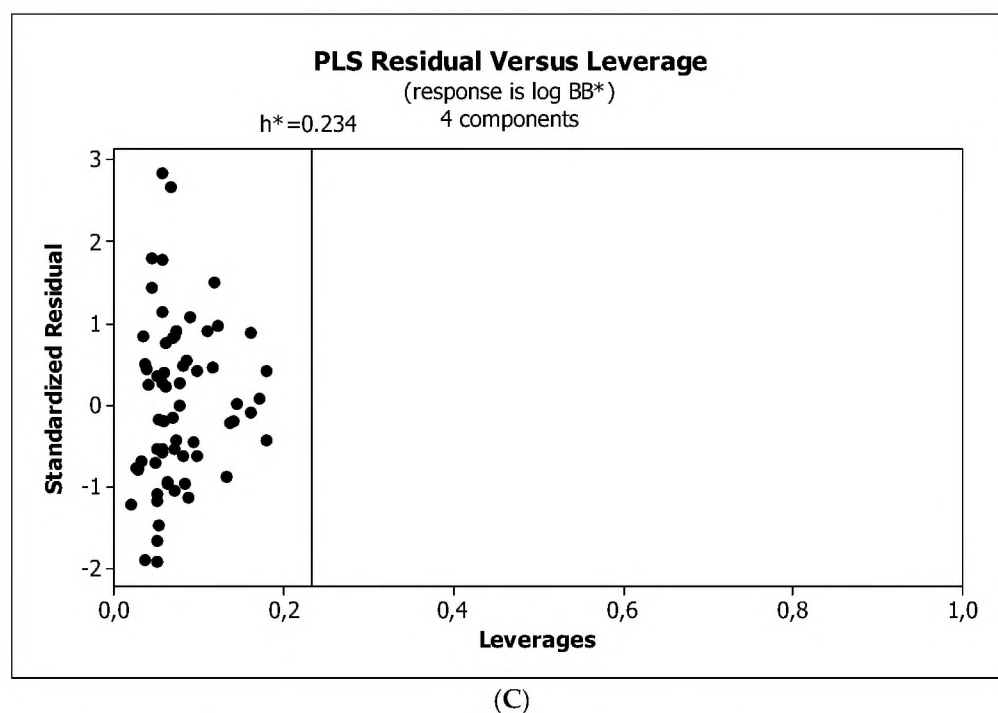

(A)

Figure 5. *Cont.*

(B)

(C)

Figure 5. (**A**) Model M11: the response plot. (**B**) Model M11: the standardized coefficients plot. (**C**) Model M11: Williams plot.

(A)

(B)

Figure 6. *Cont.*

(C)

Figure 6. (**A**) Model M12: the response plot. (**B**) Model M12: the standardized coefficients plot. (**C**) Model M12: Williams plot.

3.4. Interpretation of Descriptors

3.4.1. Lipophilicity

All derived models predict an increase in the BBB permeation with an increase of the substance lipophilicity. Thus, lipophilic compounds have a greater BBB permeability than hydrophilic. However, this influence is not indicated as dominant. Lipophilicity is undoubtedly an important parameter affecting BBB penetration, and it is the base parameter used in different QSARs modeling. Generally, transport of small molecules through membranes occurs via passive diffusion: a molecule dissolves in the phospholipid bilayer, diffuses across it, and then dissolves in the aqueous solution at the other side of the membrane. This process is closely related to the lipophilicity of the molecule: it cannot be too lipophilic, because it will not dissolve in the aqueous environment surrounding the bilayer on both sides; however, it also cannot be too hydrophilic, because it will not penetrate the lipid bilayer. Compounds investigated in this research are moderately lipophilic. Their lipophilicity, as assessed by log $P_{o/w}$, ranges from 0.868 to 4.638, for which a positive effect on log *BB* is always expected.

3.4.2. HBA

Our results indicate *HBA* as the dominant factor affecting the log *BB* values. As the number of hydrogen bond acceptors in the molecule increases, its ability to permeate the BBB decreases. Solute polarity and the ability to form hydrogen bonds increase its solubility in the aqueous environment of the membrane, and highly polar molecules do not easily enter the hydrophobic environment of the BBB. In most QSARs models, the dominant descriptor of molecules' polarity is the polar surface area. Clark [78] and Kelder et al. [33] presented linear regression between log *BB* and *PSA* only, for a group of 45 drugs. In our models, this parameter was also used (M1-M3 and M9), but due to the statistical evaluation the models including *HBA* turned out to be more accurate. However, it should be noted that the *TPSA* values of the test compounds meet the requirements for active substances. The highest *TPSA* value is 83.80 Å^2, and the number of hydrogen bond acceptors *HBA* does not exceed 10.
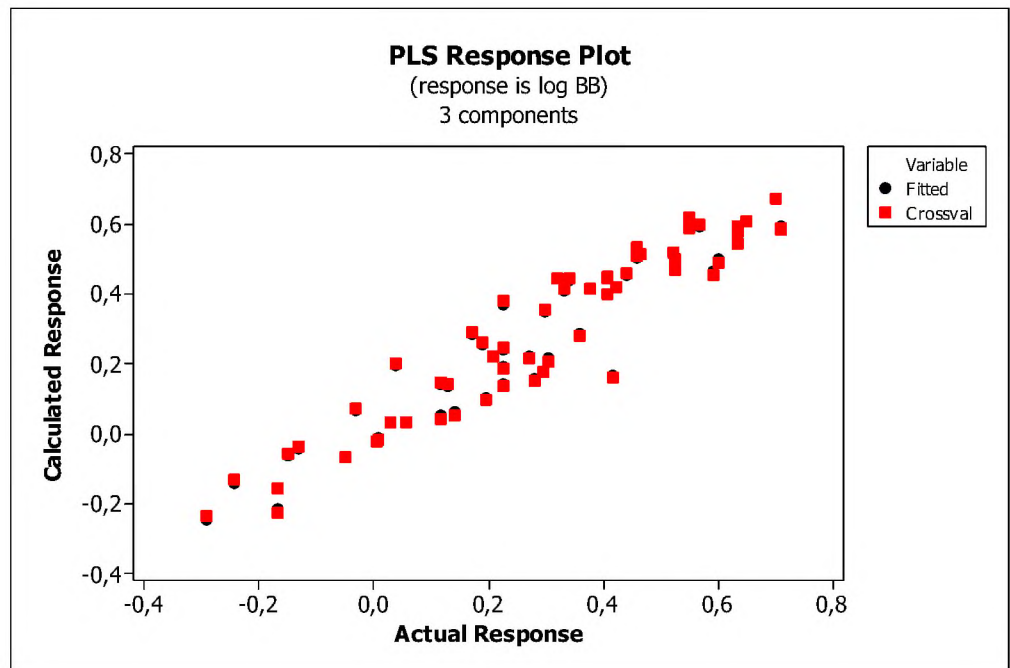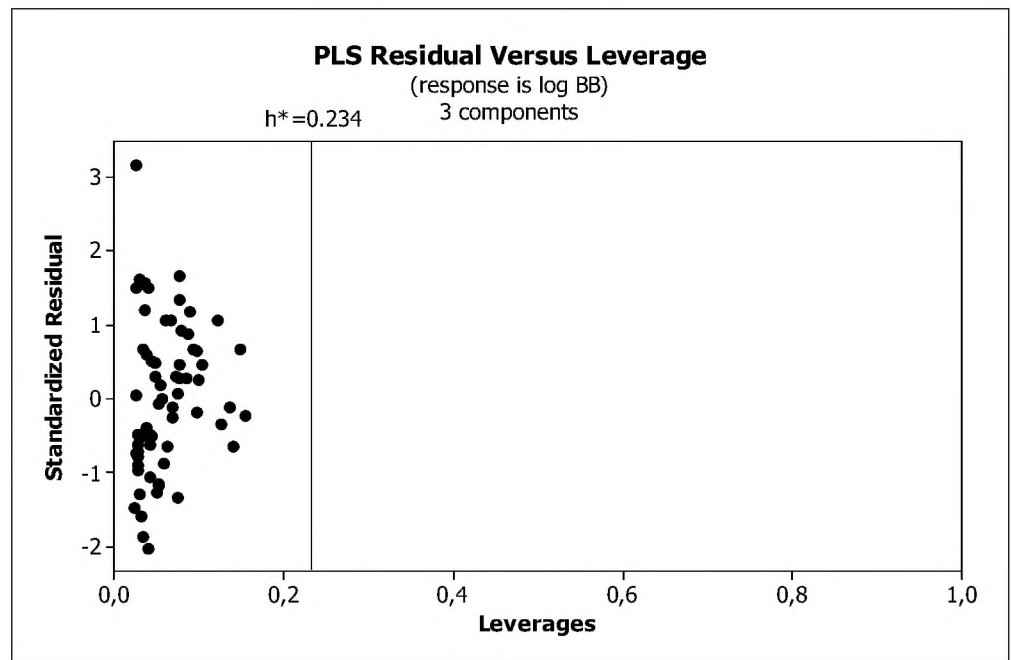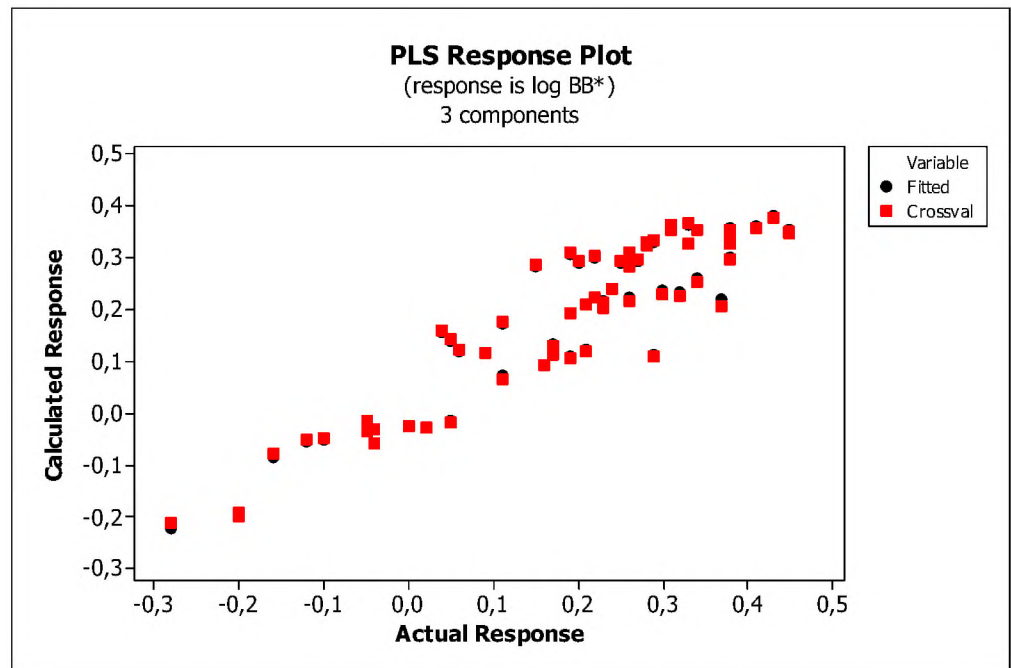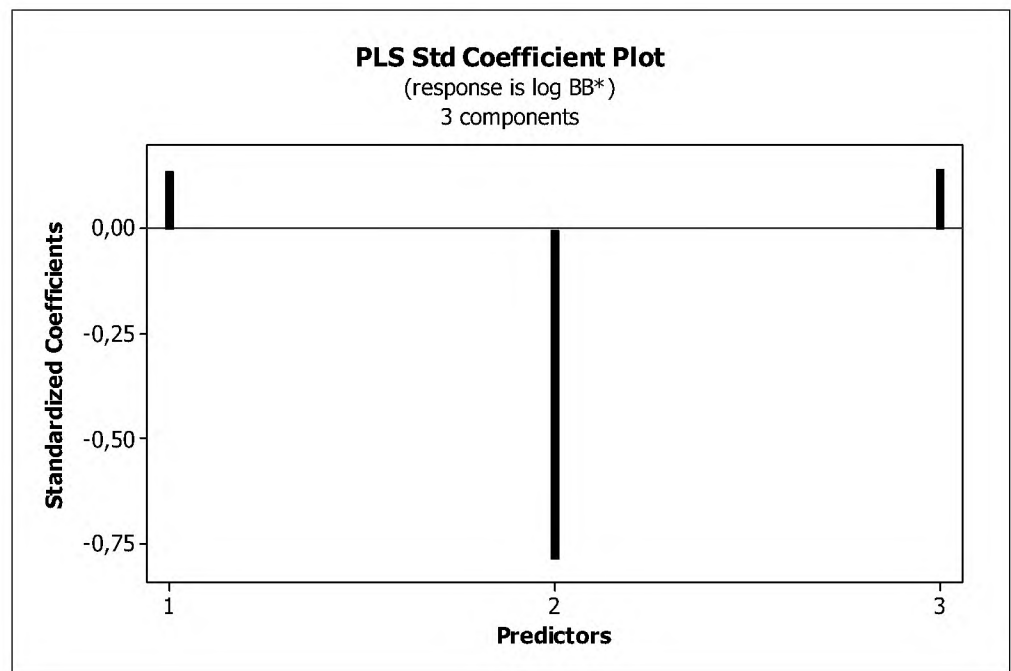
(A)
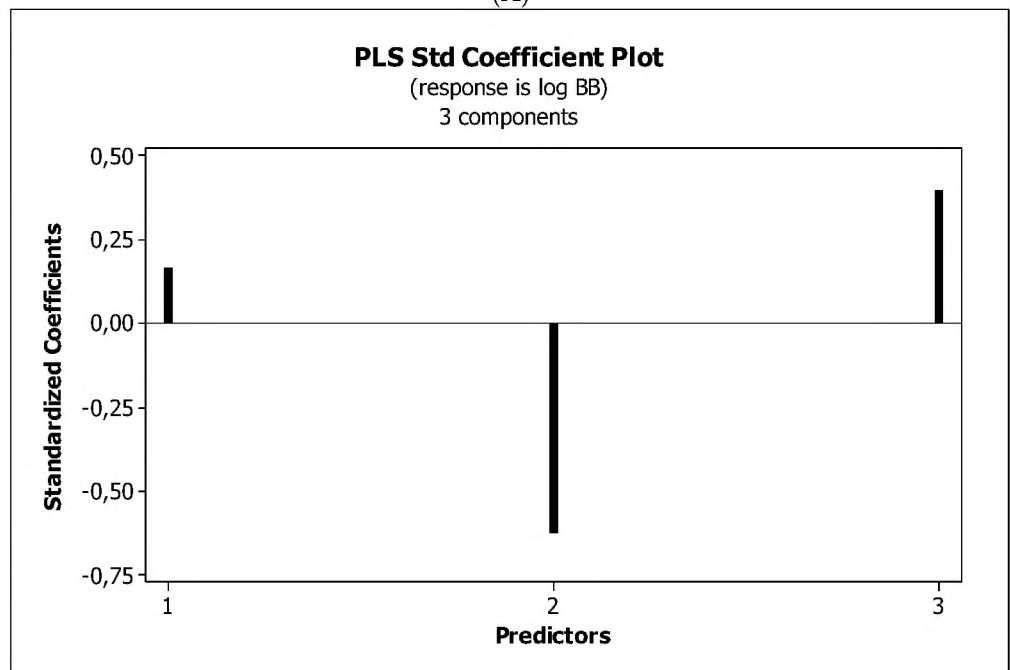
(B)

Figure 7. *Cont.*

(C)

Figure 7. (**A**) Model M5*: the response plot. (**B**) Model M5*: the standardized coefficients plot. (**C**) Model M5*: Williams plot.
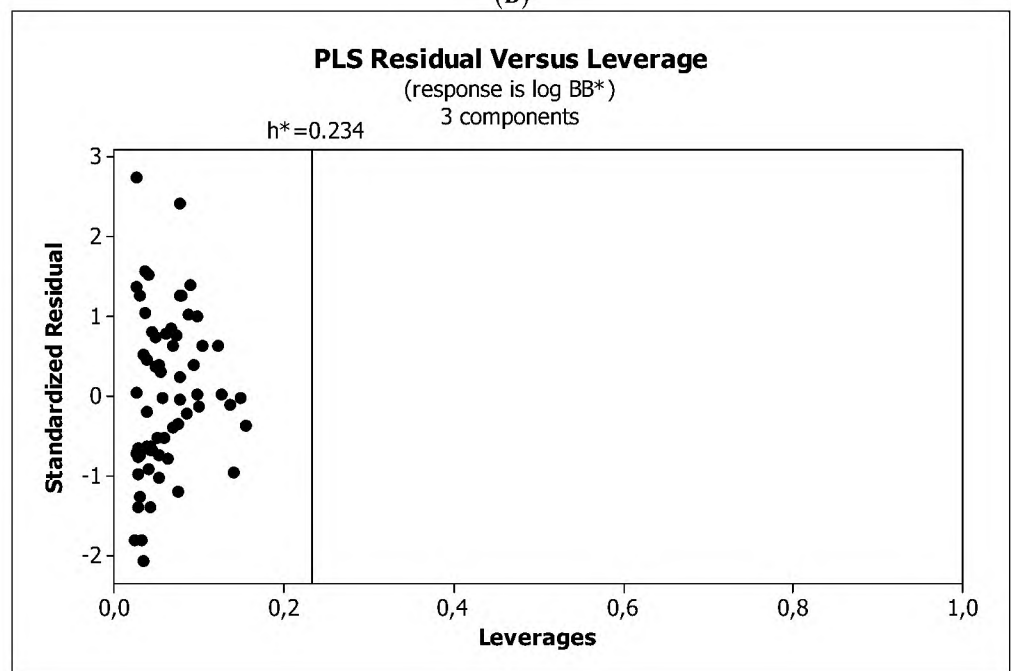

(A)

Figure 8. *Cont.*
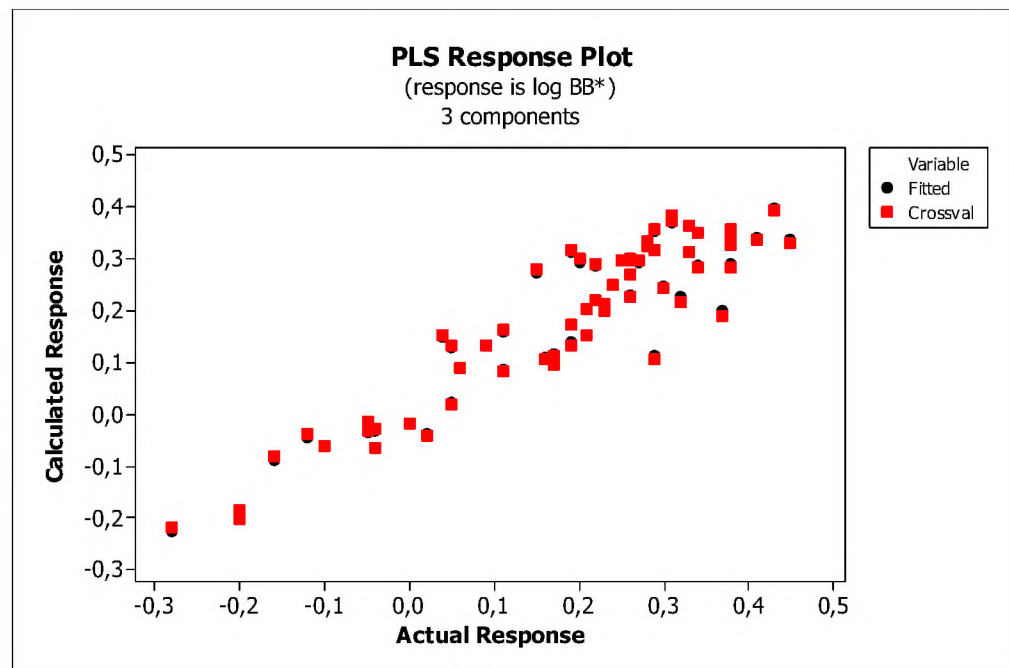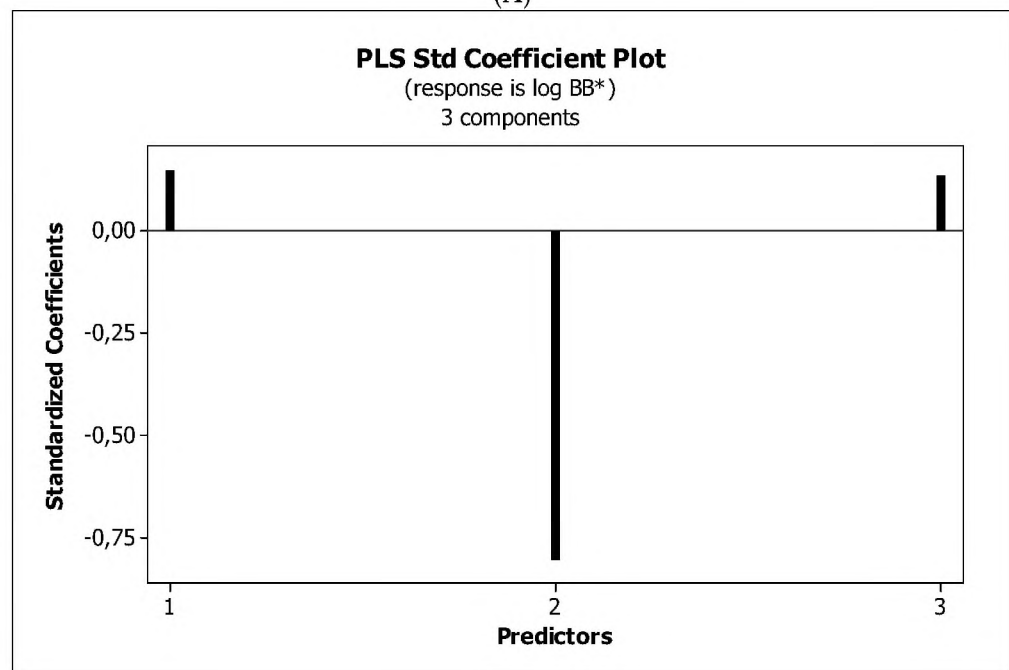
(B)

(C)

Figure 8. (**A**) Model M11*: the response plot. (**B**) Model M11*: the standardized coefficients plot. (**C**) Model M11*: Williams plot.
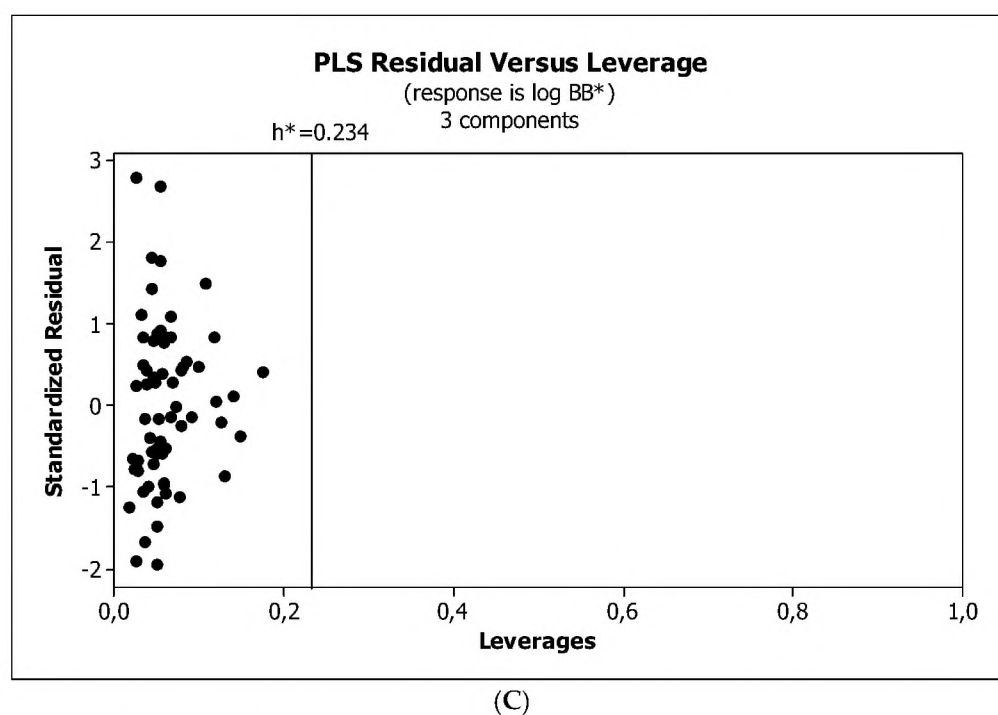
(A)

(B)

Figure 9. *Cont.*

(C)

Figure 9. (**A**) Model M12*: the response plot. (**B**) Model M12*: the standardized coefficients plot. (**C**) Model M12*: Williams plot.

3.4.3. Molecular Size

In this study, three descriptors for molecular size were proposed, i.e., molecular weight *MW*, polarizability *α*, and parachor *P*. In selected models (M5*, M11*, and M12*), *MW* and *α* and *P* turned out to be the most appropriate. The effect of molecular size on the BBB permeation (log *BB* and log *BB** values) is similar to that observed for lipophilicity, i.e., with the increase of *MW* or *P* values, the permeation of the test substances through the BBB increases. This is in contrast to the results obtained by different researchers who noticed the negative effect of molecule size on compounds' permeation through biological membranes [88–90]. The positive effect of molecular size on the log *BB* (and log *BB**) values observed herein could be explained by the partition mechanism of this process. Similarly, Platts et al. [91] obtained the positive effect of molecular size on the permeability through the skin. This relationship is a reflection of the correlation between the size of the molecule and its lipophilicity. It is especially important in the case of low molecular weights ($MW < 400 \text{ mg mol}^{-1}$), such as those in the presented research. Kouskoura et al. [12] noted that moderately increased molecular weight of the compound guarantees that its lipophilicity is sufficient to dissolve in the phospholipid bilayer and enter the BBB via passive diffusion. The same effect was observed in our previous research [92].

3.4.4. Flexibility

Research reveals that blood–brain barrier partitioning is governed not only by solute lipophilicity and polarity but also solute flexibility, solute–membrane flexibility, and solute–membrane binding. In general, ease in traversing the membrane depends on the flexibility of a compound. Limited flexibility can be considered as a merit, and higher flexibility can be proved to be a demerit [93]. The increase in BBB penetration of the solute with increasing solute flexibility was described by Iyer et al. [3]. Veber et al. [94] have found that increasing solute molecular flexibility (measured by the number of rotatable bonds) promotes a decrease in oral bioavailability. It suggests a parabolic relationship between log *BB* and molecular flexibility. That is, some "amount" of flexibility enhances log *BB*, but too much flexibility will diminish log *BB*. Our research (Figures 4B, 5B and 6B) indicates a slight and ambiguous (positive for M12 and negative for M5 and M11 models) influence of the

NRB value on the penetration of the tested substances through the blood–brain barrier. The numbers of rotatable bonds calculated for tested compounds are in the range from two to six, with average value equal to 3.2. Probably, in the case of the investigated molecules, these values are close to the maximum of the aforementioned parabolic relationship. Ultimately, *NRB* was considered a negligible factor and omitted in subsequent QSAR models.

4. Materials and Methods

4.1. Reagents and Materials

Isopropanol and acetonitrile (both HPLC grade), as well as sodium dodecyl sulfate SDS (for synthesis), were supplied from Merck (Lublin, Poland). Anhydrous citric acid ($C_6H_8O_7$) and disodium phosphate (Na_2HPO_4)—both pure—were purchased from POCh (Lublin, Poland). Deionized water was produced using the Direct-Q3 UV system (Millipore, Warsaw, Poland).

4.2. Instrumental

Shimadzu Vp (Shimadzu, Izabelin, Poland) liquid chromatographic system was used in the measurements. It was equipped with LC 10AT pump, SPD 10A UV–Vis detector, SCL 10A system controller, CTO-10 AS chromatographic oven, and Rheodyne injector valve with a 20 µL loop. A Spherisorb ODS-2 column, 125 × 4 mm i.d., 5 µm (Merck, Lublin, Poland) was applied as the stationary phase.

4.3. Chromatographic Conditions

As the mobile phases buffered, SDS mixtures (0.10; 0.105, 0.11, and 0.12 mol L^{-1}) with 7% (*v*/*v*) addition of isopropanol were used. The buffer was prepared from 0.01 mol L^{-1} solutions of disodium phosphate and citric acid, and the pH 7.4 value was fixed before mixing with an organic modifier. The flow rate was 1 mL min^{-1}. Solutes samples were dissolved in acetonitrile—c.a. 0.005 mg mL^{-1}. The compounds were detected under UV light at λ_{max} 254 nm. All measurements were carried out at 25 °C. The dead time values were measured from non-retained compound (e.g., sodium chloride) peaks. All reported *k* values are the average of at least three independent measurements.

4.4. In Silico Calculations

Molecular weight (*MW*), topological polar surface area (*TPSA*), polarizability (*α*), parachor (*P*), the numbers of hydrogen bond donors (*HBD*), acceptors (*HBA*), and rotatable bonds (*NRB*), the partition coefficient in the *n*-octanol/water system (log $P_{o/w}$), and the log *BB* values were calculated by ACD/Percepta software, version 1994–2012 (ACD/Labs, Advanced Chemistry Development, Inc., Toronto, ON, Canada).

4.5. Statistical Analysis

Linear regression (LR), multiple linear regression (MLR), and leave-one-out cross-validation (LOOcv) were conducted using the statistical software Minitab 16.2.4.0, version 1991–2004 (Minitab Inc., State College, PA, USA).

5. Conclusions

The linear quantitative structure–activity relationship models are presented for the modeling and prediction of the BBB permeation of heterocyclic drug-like molecules with promising activity. The models were produced using the multiple linear regression technique on a database that consisted of 65 recently discovered compounds. Among the different lipophilicity, polarity, electronic, and molecular size descriptors that were considered as inputs to the model, four variables were selected, i.e., micellar parameter characterizing the solutes lipophilicity log (k_m/K_{AM}), the number of hydrogen bond acceptors *HBA* connected with polarity, and parachor or molecular weight (*MW*), describing the molecular size. The rationale was to combine in the model in vitro (micellar lipophilicity parameters log (k_m/K_{AM})) and in silico (*α*, *MW*, *P*) data. The accuracy of the proposed

MLR models was illustrated using LOO cross-validation. The predictive ability of the developed models was found to be satisfactory and could be used for designing a similar set of heterocyclic compounds.

Our research confirmed that solute polarity is one of the most important properties affecting the BBB permeation. The increase of *HBA* values decreases the log *BB* (or log *BB**) values. In the QSARs models established in our studies, the number of *HBAs* was indicated as the dominant factor. The log *BB* (as well as log *BB**) values increase with micellar (chromatographic) log (k_m/K_{AM}) parameters. This means that more lipophilic drugs have a greater BBB permeability than less lipophilic. This is not the same as their CNS activity, because some compounds that are CNS-inactive may still pass through the BBB and show no activity because they do not interact with any CNS targets. Molecule size descriptors (*MW*, *P*) also increase the penetration of tested substances through the blood–brain barrier. Generally, polarity, lipophilicity, and molecular size are the most important characteristics of the investigated substances in modeling the permeation of the blood–brain barrier. The results obtained for our heterocyclic compounds indicate that less polar, more lipophilic, and bigger-size molecules partition more readily into the brain.

Our investigation shows the undoubted advantages of micellar chromatography which, combined with computational techniques, enable the prediction of BBB permeation with a high probability. This is an important achievement, especially in screening new potential drugs. It allows the reduction of unethical and expensive animal testing and respects the principles of Green Chemistry.

References

1. Eriksson, L.; Jaworska, J.; Worth, A.P.; Cronin, M.T.; McDowell, R.M.; Gramatica, P. Methods for reliability and uncertainty assessment and for applicability evaluations of classification and regression based QSARs. *Environ. Health Perspect.* **2003**, *111*, 1361–1375. [CrossRef] [PubMed]
2. Liu, P.; Long, W. Current mathematical methods used in QSAR/QSPR studies. *Int. J. Mol. Sci.* **2009**, *10*, 1978–1998. [CrossRef] [PubMed]
3. Iyer, M.; Mishra, R.; Han, Y.; Hopfinger, A.J. Predicting blood-brain barrier partitioning of organic molecules using membrane-interaction QSAR analysis. *Pharm. Res.* **2002**, *19*, 1612–1621. [CrossRef] [PubMed]
4. Adenot, M.; Lahana, R. Blood-brain barrier permeation models: Discriminating between potential CNS and non-CNS drugs including P-glycoprotein substrates. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 239–248. [CrossRef] [PubMed]
5. de Campos, L.J.; de Melo, E.B. Modeling structure–activity relationships of prodiginines with antimalarial activity using GA/MLR and OPS/PLS. *J. Mol. Graph. Model.* **2014**, *54*, 19–31. [CrossRef]
6. Kimani, N.M.; Matasyoh, J.C.; Kaiser, M.; Nogueira, M.S.; Trossini, G.H.G.; Schmidt, T.J. Complementary quantitative structure–activity relationship models for the antitrypanosomal activity of sesquiterpene lactones. *Int. J. Mol. Sci.* **2018**, *19*, 3721. [CrossRef]
7. Pourbasheer, E.; Riahi, S.; Ganjali, M.R.; Norouzi, P. Quantitative structure–activity relationship (QSAR) study of interleukin-1 receptor associated kinase 4 (IRAK-4) inhibitor activity by the genetic algorithm and multiple linear regression (GA-MLR) method. *J. Enzyme Inhib. Med. Chem.* **2010**, *25*, 844–853. [CrossRef]
8. Tugcu, G.; Saçan, M.T.; Vračko, M.; Novič, M.; Minovski, N. QSTR modelling of the acute toxicity of pharmaceuticals to fish. *SAR QSAR Environ. Res.* **2012**, *23*, 297–310. [CrossRef]

9. Ciura, K.; Belka, M.; Kawczak, P.; Bączek, T.; Markuszewski, M.J.; Nowakowska, J. Combined computational-experimental approach to predict blood–brain barrier (BBB) permeation based on "green" salting-out thin layer chromatography supported by simple molecular descriptors. *J. Pharm. Biomed. Anal.* **2017**, *143*, 214–221. [CrossRef]

10. Liu, R.; Sun, H.; So, S.-S. Development of quantitative structure-property relationship models for early ADME evaluation in drug discovery. 2. Blood-brain barrier penetration. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 1623–1632. [CrossRef]

11. Ajay; Bemis, G.W.; Murcko, M.A. Designing libraries with CNS activity. *J. Med. Chem.* **1999**, *42*, 4942–4951. [CrossRef] [PubMed]

12. Kouskoura, M.G.; Piteni, A.I.; Markopoulou, C.K. A new descriptor via bio-mimetic chromatography and modeling for the blood brain barrier (Part II). *J. Pharm. Biomed. Anal.* **2019**, *164*, 808–817. [CrossRef] [PubMed]

13. Goldstein, G.W.; Betz, A.L. The blood-brain barrier. *Sci. Am.* **1986**, *255*, 74–83. [CrossRef] [PubMed]

14. Pardridge, W.M. CNS drug design based on principles of blood brain barrier transport. *J. Neurochem.* **1998**, *70*, 1781–1792. [CrossRef]

15. Begley, D.J. The blood-brain barrier: Principles for targeting peptides and drugs to the central nervous system. *J. Pharm. Pharmacol.* **1996**, *48*, 136–140. [CrossRef]

16. Mouritsen, O.G.; Jorgensen, K. A new look at lipid membrane structure in relation to drug research. *Pharm. Res.* **1998**, *15*, 1507–1519. [CrossRef]

17. Sugano, K.; Kansy, M.; Artursson, P.; Avdeef, A.; Bendels, S.; Di, L.; Ecker, G.F.; Faller, B.; Fischer, H.; Gerebtzoff, G.; et al. Coexistence of passive and carrier-mediated processes in drug transport. *Nat. Rev. Drug Discov.* **2010**, *9*, 597–614. [CrossRef]

18. Sugiyama, Y.; Kusuhara, H.; Suzuki, H. Kinetic and biochemical analysis of carrier-mediated efflux of drugs through the blood-brain and blood-cerebrospinal fluid barriers: Importance in the drug delivery to the brain. *J. Cont. Rel.* **1999**, *62*, 179–186. [CrossRef]

19. Seddon, A.M.; Casey, D.; Law, R.V.; Gee, A.; Templera, R.H.; Cesab, O. Drug interactions with lipid membranes. *Chem. Soc. Rev.* **2009**, *38*, 2509–2519. [CrossRef]

20. Wolak, D.J.; Thorne, R.G. Diffusion of macromolecules in the brain: Implications for drug delivery. *Mol. Pharm.* **2013**, *10*, 1492–1504. [CrossRef]

21. Banks, W.A. Characteristics of compounds that cross the blood-brain barrier. *BMC Neurol.* **2009**, *9* (Suppl. 1), S3. [CrossRef] [PubMed]

22. Hemmateenejad, B.; Miri, R.; Safarpour, M.A.; Mehdipour, A.R. Accurate prediction of the blood-brain partitioning of a large set of solutes using ab initio calculations and genetic neural network modeling. *J. Comput. Chem.* **2006**, *27*, 1125–1135. [CrossRef] [PubMed]

23. Winkler, D.A.; Burden, F.R. Modeling blood–brain barrier partitioning using Bayesian neural nets. *J. Mol. Graphics Model.* **2004**, *22*, 499–505. [CrossRef] [PubMed]

24. Luco, M. Prediction of the brain–blood distribution of a large set of drugs from structurally derived descriptors using partial least-squares (PLS) modeling. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 396–404. [CrossRef]

25. Liu, X.; Tu, M.; Kelly, R.S.; Chen, C.; Smith, B.J. Development of a computational approach to predict blood-brain barrier permeability. *Drug Metab. Dispos.* **2004**, *32*, 132–139. [CrossRef]

26. Lombardo, F.; Blake, J.F.; Curatolo, W. Computation of brain-blood partitioning of organic solutes via free-energy calculations. *J. Med. Chem.* **1996**, *39*, 4750–4755. [CrossRef]

27. Keserü, G.M.; Molnar, L. High-throughput prediction of blood-brain partitioning: A thermodynamic approach. *J. Chem. Inf. Comput. Sci.* **2001**, *41*, 120–128. [CrossRef]

28. Norinder, U.; Sjöberg, P.; Osterberg, T. Theoretical calculations and prediction of brain-blood partitioning of organic solutes using MolSurf parametrization and PLS statistics. *J. Pharm. Sci.* **1998**, *88*, 815–821. [CrossRef]

29. Crivori, P.; Cruciani, G.; Carrupt, P.-A.; Testa, B. Predicting blood-brain permeation from three-dimensional molecular structure. *J. Med. Chem.* **2000**, *43*, 2204–2216. [CrossRef]

30. Osterberg, T.; Norinder, U. Prediction of polar surface area and drug transport processes using simple parameters and PLS statistics. *J. Chem. Inf. Comput. Sci.* **2000**, *40*, 1408–1411. [CrossRef]

31. Rose, K.; Hall, L.H.; Kier, L.B. Modeling blood-brain barrier partitioning using the electrotopological state. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 651–666. [CrossRef] [PubMed]

32. Basak, S.C.; Gute, B.D.; Drewes, L.R. Predicting blood brain transport of drugs: A computational approach. *Pharm. Res.* **1996**, *13*, 775–778. [CrossRef] [PubMed]

33. Kelder, J.; Grootenhuis, P.D.; Bayada, D.M.; Delbressine, L.P.C.; Ploemen, J.P. Polar molecular surface as dominating determinant for oral absorption and brain penetration of drugs. *Pharm. Res.* **1999**, *16*, 1514–1519. [CrossRef] [PubMed]

34. Ertl, P.; Rohde, B.; Selzer, P. Fast calculation of molecular polar surface area as a sum of fragment based contributions and its application to the prediction of drug transport properties. *J. Med. Chem.* **2000**, *43*, 3714–3717. [CrossRef]

35. Abraham, M.H.; Chadha, H.S.; Mitchell, R.C. Hydrogen bonding. 33. Factors that influence the distribution of solutes between blood and brain. *J. Pharm. Sci.* **1994**, *83*, 1257–1268. [CrossRef]

36. van de Waterbeemd, H.; Camenish, G.; Folkers, G.; Chretien, J.R.; Raevsky, O.A. Estimation of blood-brain barrier crossing of drugs using molecular size and shape, and H-bonding descriptors. *J. Drugs Target.* **1998**, *6*, 151–165. [CrossRef]

37. Kaliszan, R. QSRR Quantitative structure—(chromatographic) retention relationships. *Chem. Rev.* **2007**, *107*, 3212–3246. [CrossRef]

38. Dąbrowska, M.; Komsta, Ł.; Krzek, J.; Kokoszka, K. Lipophilicity study of eight cephalosporins by reversed-phase thin-layer chromatographic method. *Biomed. Chromatogr.* **2015**, *29*, 1759–1768. [CrossRef]

39. Kempińska, D.; Chmiel, T.; Kot-Wasik, A.; Mróz, A.; Mazerska, Z.; Namieśnik, J. State of the art and prospects of methods for determination of lipophilicity of chemical compounds. *TrAC Trends Anal. Chem.* **2019**, *113*, 54–73. [CrossRef]

40. Kurbatova, S.V.; Saifutdinov, B.R.; Larionov, O.G.; Meshkovaya, V.V. The influence of the structure of some aromatic heterocyclic derivatives on their retention in reversed-phase high-performance liquid chromatography. *Russ. J. Phys. Chem.* **2009**, *83*, 471–477. [CrossRef]

41. Sagandykova, G.N.; Pomastowski, P.P.; Kaliszan, R.; Buszewski, B. Modern analytical methods for consideration of natural biological activity. *TrAC Trends Anal. Chem.* **2018**, *109*, 198–213. [CrossRef]

42. Milošević, N.P.; Stojanović, S.Z.; Penov-Gaši, K.; Perišić-Janjić, N.; Kaliszan, R. Reversed- and normal-phase liquid chromatography in quantitative structure retention-property relationships of newly synthesized seco-androstene derivatives. *J. Pharm. Biomed. Anal.* **2014**, *88*, 636–642. [CrossRef] [PubMed]

43. Héberger, K. Quantitative structure-(chromatographic) retention relationships. *J. Chromatogr. A* **2007**, *1158*, 273–305. [CrossRef] [PubMed]

44. Andrić, F.; Héberger, K. Towards better understanding of lipophilicity: Assessment of in silico and chromatographic log P measures for pharmaceutically important compounds by nonparametric rankings. *J. Pharm. Biomed. Anal.* **2015**, *115*, 183–191. [CrossRef]

45. Valko, K.; Nunhuck, S.; Bevan, C.; Abraham, M.H.; Reynolds, D.P. Fast gradient HPLC method to determine compounds binding to human serum albumin. Relationships with octanol/water and immobilized artificial membrane lipophilicity. *J. Pharm. Sci.* **2003**, *92*, 2236–2248.

46. Valko, K. Lipophilicity and biomimetic properties measured by HPLC to support drug discovery. *J. Pharm. Biomed. Anal.* **2016**, *130*, 35–54. [CrossRef]

47. Ciura, K.; Dziomba, S. Application of separation methods for in vitro prediction of blood–brain barrier permeability—The state of the art. *J. Pharm. Biomed. Anal.* **2020**, *177*, 112891. [CrossRef]

48. Milošević, N.; Janjić, N.; Milić, N.; Milanović, M.; Popović, J.; Antonović, D. Pharmacokinetics and toxicity predictors of new s-triazines, herbicide candidates, in correlation with chromatographic retention constants. *J. Agric. Food Chem.* **2014**, *62*, 8579–8585. [CrossRef]

49. Tsopelas, F.; Giaginis, C.; Tsantili-Kakoulidou, A. Lipophilicity and biomimetic properties to support drug discovery. *Expert Opin. Drug Discov.* **2017**, *12*, 885–896. [CrossRef]

50. Russo, G.; Grumetto, L.; Szucs, R.; Barbato, F.; Lynen, F. Screening therapeutics according to their uptake across the blood-brain barrier: A high throughput method based on immobilized artificial membrane liquid chromatography-diode-array-detection coupled to electrospray-time-of-flight mass spectrometry. *Eur. J. Pharm. Biopharm.* **2018**, *127*, 72–84. [CrossRef]

51. Stergiopoulos, C.; Tsopelas, F.; Valko, K.; Ochsenkühn-Petropoulou, M. The use of biomimetic chromatography to predict acute aquatic toxicity of pharmaceutical compounds. *Toxicol. Environ. Chem.* **2022**, *104*, 1–19. [CrossRef]

52. Escuder-Gilabert, L.; Molero-Monfort, M.; Villanueva- Camañas, R.M.; Sagrado, S.; Medina-Hernández, M.J. Potential of biopartitioning micellar chromatography as an in vitro technique for predicting drug penetration across the blood-brain barrier. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2004**, *807*, 193–201. [CrossRef] [PubMed]

53. Ruiz-Ángel, M.; Garcia-Álvarez-Coque, M.; Berthod, A. New insights and recent developments in micellar liquid chromatography. *Sep. Pur. Rev.* **2009**, *38*, 45–96. [CrossRef]

54. Rambla-Alegre, M. Basic principles of MLC. *Chromatogr. Res. Int.* **2012**, *2012*, 898520. [CrossRef]

55. Kalyankar, T.M.; Kulkarni, P.D.; Wadher, S.J.; Pekamwar, S.S. Applications of micellar liquid chromatography in bioanalysis: A review. *J. Appl. Pharm. Sci.* **2014**, *4*, 128–134.

56. Tsopelas, F.; Danias, P.; Pappa, A.; Tsantili-Kakoulidou, A. Biopartitioning micellar chromatography under different conditions: Insight into the retention mechanism and the potential to model biological processes. *J. Chromatogr. A* **2020**, *1621*, 461027. [CrossRef]

57. Foley, J.P. Critical compilation of solute-micelle binding constants and related parameters from micellar liquid chromatographic measurements. *Anal. Chim. Acta* **1990**, *231*, 237–247. [CrossRef]

58. Janicka, M.; Sztanke, M.; Sztanke, K. Reversed-phase liquid chromatography with octadecylsilyl, immobilized artificial membrane and cholesterol columns in correlation studies with in silico biological descriptors of newly synthesized antiproliferative and analgesic active compounds. *J. Chromatogr. A* **2013**, *1318*, 92–101. [CrossRef]

59. Sztanke, M.; Tuzimski, T.; Janicka, M.; Sztanke, K. Structure-retention behaviour of biologically active fused 1,2,4-triazinones—Comparison with in silico molecular properties. *Eur. J. Pharm. Sci.* **2015**, *68*, 114–126. [CrossRef]

60. Sztanke, M.; Rzymowska, J.; Janicka, M.; Sztanke, K. Synthesis, structure confirmation, identification of in vitro antiproliferative activities and correlation of determined lipophilicity parameters with in silico bioactivity descriptors of two novel classes of fused azaisocytosine-like congeners. *Arabian J. Chem.* **2019**, *12*, 5302–5324. [CrossRef]

61. Sztanke, M.; Sztanke, K. 3-(2-Phenylethyl)-8-aryl-7,8-dihydroimidazo[2,1-c][1,2,4]triazin-4(6H)-ones, Method for Obtaining Them and Medical Application. Polish Patent PL 224678, 31 January 2017.

62. Sztanke, M.; Sztanke, K. 3-Ethyl-8-aryl-7,8-dihydroimidazo[2,1-c][1,2,4]triazin-4(6H)-ones, Method for Obtaining Them and Medical Application. Polish Patent PL 224679, 31 January 2017.

63. Sztanke, K.; Sztanke, M. Ethyl 2-(4-oxo-4,6,7,8-tetrahydroimidazo[2,1-c][1,2,4]triazin-3-yl)acetates, Method for Obtaining Them and Medical Application. Polish Patent PL 219424, 30 April 2015.

64. Sztanke, M.; Rzymowska, J.; Sztanke, K. Synthesis, structure elucidation and in vitro anticancer activities of novel derivatives of diethyl (2E)-2-[(2E)-(1-arylimidazolidin-2-ylidene)hydrazono]succinate and ethyl(4-oxo-8-aryl-4,6,7,8-tetrahydroimidazo[2,1-c][1,2,4]triazin-3-yl)acetate. *Bioorg. Med. Chem.* **2013**, *21*, 7465–7480. [CrossRef] [PubMed]

65. Sztanke, M.; Sztanke, K.; Rajtar, B.; Świątek, Ł.; Boguszewska, A.; Polz-Dacewicz, M. The influence of some promising fused azaisocytosine-containing congeners on zebrafish (*Danio rerio*) embryos/larvae and their antihaemolytic, antitumour and antiviral activities. *Eur. J. Pharm. Sci.* **2019**, *132*, 34–43. [CrossRef] [PubMed]

66. Sztanke, K.; Tuzimski, T.; Sztanke, M.; Rzymowska, J.; Pasternak, K. Synthesis, structure elucidation, determination of the lipophilicity and identification of antitumour activities in vitro of novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-c][1,2,4]triazin-4(6H)-ones with a low cytotoxicity towards normal human skin fibroblast cells. *Bioorg. Med. Chem.* **2011**, *19*, 5103–5116. [PubMed]

67. Sztanke, K.; Sztanke, M.; Pasternak, K. 3-(2-Furanyl)-7,8-dihydroimidazo[2,1-c][1,2,4]triazin-4(6H)-ones Substituted with Mono- or Dichlorophenyl and Process for the Preparation Thereof. Polish Patent PL 212442, 31 October 2012.

68. Sztanke, K.; Sztanke, M.; Pasternak, K. Derivatives of 3-(2-furanyl)-7,8-dihydroimidazo[2,1-c][1,2,4]triazin-4(6H)-one Substituted with Phenyl, Alkylphenyl, Alkoxyphenyl and Process for the Preparation Thereof. Polish Patent PL 212447, 31 October 2012.

69. Sztanke, K. New 8-aryl-3-phenyl-6,7-dihydro-4H-imidazo[2,1-c][1,2,4]triazine-4-ones and Methods for Their Manufacture. Polish Patent PL 199750, 31 October 2008.

70. Sztanke, K.; Pasternak, K.; Sztanke, M.; Kandefer-Szerszeń, M.; Kozioł, A.E.; Dybała, I. Crystal structure, antitumour and antimetastatic activities of disubstituted fused 1,2,4-triazinones. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5095–5100. [CrossRef] [PubMed]

71. Tuzimski, T.; Sztanke, K. Retention data for some carbonyl derivatives of imidazo[2,1-c][1,2,4]triazine in reversed-phase systems in TLC and HPLC and their use for determination of lipophilicity. Part 1. Lipophilicity of 8-aryl-3-phenyl-6,7-dihydro-4H-imidazo[2,1-c][1,2,4]triazin-4-ones. *J. Planar Chromatogr.* **2005**, *18*, 274–281.

72. Bartyzel, A.; Sztanke, M.; Sztanke, K. Thermal behaviour of antiproliferative active 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-c][1,2,4]triazin-4(6H)-ones. *J. Therm. Anal. Calorim.* **2017**, *130*, 1541–1551. [CrossRef]

73. Stępniowska, A.; Sztanke, M.; Tuzimski, T.; Korolczuk, M.; Sztanke, K. A simple stripping voltammetric method for the determination of a new anticancer prodrug in serum. *Biosens. Bioelectron.* **2017**, *94*, 584–588. [CrossRef]

74. Kozak, J.; Tyszczuk-Rotko, K.; Sadok, I.; Sztanke, K.; Sztanke, M. Application of screen-printed sensor modified with carbon nanofibers for the voltammetric analysis of an anticancer disubstituted fused triazinone. *Int. J. Mol. Sci.* **2022**, *23*, 2429. [CrossRef]

75. Janicka, M.; Sztanke, M.; Sztanke, K. Predicting the blood-brain barrier permeability of new drug-like compounds via HPLC with various stationary phases. *Molecules* **2020**, *25*, 487. [CrossRef]

76. Janicka, M.; Śliwińska, A. Quantitative retention (structure)—Activity relationships in predicting the pharmaceutical and toxic properties of potential pesticides. *Molecules* **2022**, *27*, 3599. [CrossRef]

77. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3–25. [CrossRef]

78. Clark, D.E. In silico prediction of blood−brain barrier permeation. *Drug Discov. Today* **2003**, *15*, 927–933. [CrossRef] [PubMed]

79. Roy, K.; Kar, S.; Das, R.N. *A Primer on QSAR/QSPR Modeling: Fundamental Concepts*; Springer Briefs in Molecular Science; Springer: Berlin/Heidelberg, Germany, 2015; pp. 1–35.

80. Roy, K.; Kar, S.; Das, R.N. *Understanding the Basics of QSAR for Applications in Pharmaceutical Sciences and Risk Assessment*; Academic Press: Cambridge, MA, USA, 2015; pp. 1–46.

81. Hamadache, M.; Benkortbi, O.; Hanini, S.; Amrane, A. QSAR modeling in ecotoxicological risk assessment: Application to the prediction of acute contact toxicity of pesticides on bees (*Apis mellifera* L.). *Environ. Sci. Pollut. Res.* **2018**, *25*, 896–907. [CrossRef] [PubMed]

82. Hamadache, M.; Benkortbi, O.; Hanini, H.; Amrane, A.; Khaouane, L.; Si Moussa, C. A quantitative structure activity relationship for acute oral toxicity of pesticides on rats: Validation, domain of application and prediction. *J. Hazard. Mater.* **2016**, *303*, 28–40. [CrossRef] [PubMed]

83. Sawant, S.D.; Nerkar, A.G.; Pawar, N.D.; Velapure, A.V. Design, synthesis, QSAR studies and biological evaluation of novel triazolopiperazine based β-amino amides as dipeptidyl peptidase-IV (DPP-IV) inhibitors: Part-II. *Int. J. Pharm. Pharm. Sci.* **2014**, *6*, 812–817.

84. Clementi, M.; Clementi, S.; Fornaciari, M.; Orlandi, F.; Romano, B. The GOLPE procedure for predicting olive crop production from climatic parameters. *J. Chemom.* **2001**, *15*, 397–404. [CrossRef]

85. Chen, J.W.; Li, X.H.; Yu, H.Y.; Wang, Y.N.; Qiao, X.L. Progress and perspectives of quantitative structure–activity relationships used for ecological risk assessment of toxic organic compounds. *Sci. China Ser. B-Chem.* **2008**, *51*, 593–606. [CrossRef]

86. Organization for Economic Co-Operation and Development, Guidance Document on the Validation of (Quantitative) Structure–Activity Relationship[(Q)SAR] Models. March 2007. Available online: https://www.oecd.org/env/guidance-document-on-the-validation-of-quantitative-structure-activity-relationship-q-sar-models-9789264085442-en.htm (accessed on 13 April 2022).

87. Saaidpour, S.; Bahmani, A.; Rostami, A. Prediction the normal boiling points of primary, secondary and tertiary liquid amines from their molecular structure descriptors. *CMST* **2015**, *21*, 201–210. [CrossRef]

88. Kaliszan, R.; Markuszewski, M. Brain-blood distribution described by a combination of partition coefficients and molecular mass. *Int. J. Pharm.* **1996**, *45*, 9–16. [CrossRef]

89. Testa, B.; Crivori, P.; Reist, M.; Carrupt, P.A. The influence of lipophilicity on the pharmacokinetic behavior of drugs: Concepts and examples. *Persp. Drug Discov. Des.* **2000**, *19*, 179–211. [CrossRef]

90. Levin, V.A. Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability. *J. Med. Chem.* **1980**, *23*, 682–684. [CrossRef] [PubMed]

91. Platts, J.A.; Abraham, M.H.; Hersey, A.; Butina, D. Estimation of molecular linear free energy relationship descriptors. 4. Correlation and prediction of cell permeation. *Pharm. Res.* **2000**, *17*, 1013–1018. [CrossRef] [PubMed]

92. Janicka, M.; Mycka, A.; Sztanke, M.; Sztanke, K. Predicting pharmacokinetic properties of potential anticancer agents via their chromatographic behavior on different reversed phase materials. *Int. J. Mol. Sci.* **2021**, *22*, 4257. [CrossRef] [PubMed]

93. Pan, D.; Iyer, M.; Liu, J.; Li, Y.; Hopfinger, A.J. Constructing optimum blood brain barrier QSAR models using a combination of 4D-molecular similarity measures and cluster analysis. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 2083–2098. [CrossRef]

94. Veber, D.F.; Johnson, S.R.; Cheng, H.-Y.; Smith, B.R.; Ward, K.W.; Kopple, K.D. Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* **2002**, *45*, 2615–2623. [CrossRef] [PubMed]