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Validation of olive-castor oil (OL-C) blend as a bio-membrane model

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The need for suitable *in vitro* biological membrane models for accurate prediction of *in vivo* biodisposition of potential drug molecules is critical in early-stage drug discovery science. The Olive-Castor oil (OL-C) model was previously reported as possessing a fairly complex architecture compared to routinely used models like octadecylsilane (ODS). However, further studies to validate the bio-relevance of its lipophilicity characterization were recommended. Therefore, to further validate its performance in bio-membrane simulation on a planar chromatographic platform, the retention characteristics of some opioids with similar pharmacophores were determined using a methanol/water binary mixture of varying compositions as the mobile phase on OL-C and ODS platforms. Their limit of agreement for lipophilicity determination was assessed by a Bland–Altman plot, and correlation with *in vivo*-related biological parameters and calculated molecular descriptors was carried out to evaluate bio-relevance. The lipophilicity indices on ODS were higher than those on OL-C for all the evaluated opioid compounds. The Bland–Altman plot revealed a low level of agreement between the two lipophilicity determinations. OL-C gave higher correlations with all the computed molecular descriptors, pharmacokinetic parameters (AUC: 0.41 *vs.* 0.23; C_{max}: 0.22 *vs.* 0.054), and the *in vivo*-related bioactivity descriptors (Plasma Protein Binding (PPB): 0.43 *vs.* 0.29; Human Intestinal Absorption (HIA): 0.62 *vs.* 0.43; Blood–Brain Barrier (BBB) penetration: 0.33 *vs.* 0.22). OL-C outperformed the widely used ODS as a potential biomimetic platform for lipophilicity profiling of selected small-molecule drugs. This has the prospect of significantly improving accuracy in medicinal chemistry and drug discovery science. Thus, a wider validation study is warranted.

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1. Introduction

Bio-membrane modeling has been a subject of interest in the past few decades to gain critical insights into the molecular dynamics and organization that influence cell function. In medicinal chemistry and drug discovery science, this is of notable importance because drug action and relative efficacy are controlled by a drug's perfusion of the bio-membrane. The molecular architecture and molecular organization of the bio-membrane – comprising a bilayer of phospholipids, integral and peripheral glycoproteins, glycolipids, sterols, *etc.* – are intrinsically more complex than in models used in the past to evaluate its functionality; and it is challenging to adequately mimic.[1] Therefore, there is a need for a continuous search for reliable *in vitro* models of comparable molecular attributes to ensure good mimicry of *in vivo* bio-molecular functionality and interactions.

Lipophilicity is a crucial physicochemical parameter for exploring biomembrane attributes such as permeability and electrostatic interactions, and it is essential in medicinal chemistry for evaluating the potency and the pharmacokinetic and toxicological profiles of potential drug candidates.[2] While chromatographic techniques have offered reliable opportunities for the estimation of compound lipophilicity, their use in drug discovery has been weakened by poor and unreliable prediction of *in vivo* passive diffusion of the drugs across the bio-membrane.[3] Such low reliability of these chromatographic models could be attributed to the less complex dynamics of solute partitioning on account of their surface chemistry when compared to the bio-membrane complexity. The low reliability of permeability prediction of chemical compounds underscores the urgent need for standardization and validation of high-throughput procedures for lipophilicity determination.[4] This is corroborated by reports that the chemical architecture of chromatographic stationary phases and hence the nature of solute interactions significantly influence the retention parameters,[5] and for bio-membrane modeling, these stationary

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phases must be carefully chosen to ensure the close mimic of the intermolecular interactions operable within the biological membrane.[6] A number of stationary phases or artificial membranes comprised of phospholipids, liposomes, lipopolysaccharides, *etc.*[7] have been reported to be used in simulating bio-membrane amphiphilicity, albeit in liquid chromatography.

Idowu *et al.* reported a plant-derived Leucaena oil film as a reliable biomimetic stationary phase for planar chromatographic analysis.[1] In addition to this, a new, oil-based stationary film comprised of a 1.25% equivolume mixture of olive/castor oil (OL-C) in *n*-hexane was optimized for use as a potential biomimetic membrane by Adeyemo *et al.*[8] The usefulness of this new stationary surface is dependent on the validity of its measurement. Validity is described as "the extent to which a measure or set of measures correctly represents the concept of study – the degree to which it is free from any systematic or non-random error".[9] This study is a further evaluation of the validity of this lipid film in lipophilicity determination and its suitability for predicting *in vivo* drug absorption.

2. Materials and methods

2.1. Materials

OL-C (1.25%), castor oil (Technical grade; Bell, UK, with acid value 0.61), olive oil (Technical grade; Goya, Spain, with acid value of 0.95), octadecylsilane plate (Merck, Germany), methanol (Merck, Germany), *n*-hexane (Analar, BDH), distilled water, and filter papers. The model opioid compounds ("opioid" here refers to both opiate and opioid compounds) in this study were codeine, pentazocine, dextromethorphan, tramadol, naloxone and naltrexone (Fig. 1).

2.2. Equipment

UV lamp 254/365 nm (Gallenkamp, UK), water bath, and drying oven (Astell Hearson PBS 040, England).

2.3. Methods

2.3.1. Lipophilicity profiling of model compounds. The lipophilicity evaluation of the six (6) model compounds comprising opioid drugs was carried out using reversed-phase thin layer chromatographic procedure on silica gel plate GF_{254} (5 × 10 cm) impregnated with 1.25% of an equi-mixture of commercially available olive oil and castor oil (OL-C), and octadecylsilane chemical bonded C-18 plates as stationary phases, as previously reported by Adeyemo *et al.* that also described the composition and preparation of the OL-C stationary phase.[8] Binary mixtures of methanol/water of varying compositions were used as mobile phases. The biodevice was always freshly prepared and not stored before use. The methanolic solutions of the model compounds were spotted on the respective stationary phases, air dried and developed in the saturated chromatographic tanks by ascending technique. The developed plates were thereafter air-dried, visualized under the UV lamp, and the retardation factor (R_f) of the corresponding spots measured. The R_f was transformed to R_m, and plotted against the organic modifier fraction of the mobile phase to generate a linear relationship based on the equation:

$$R_m = R_{mw} \pm S\varphi$$

where φ is the organic modifier fraction, S is the slope and indicates the rate of solute partitioning into the aqueous phase,

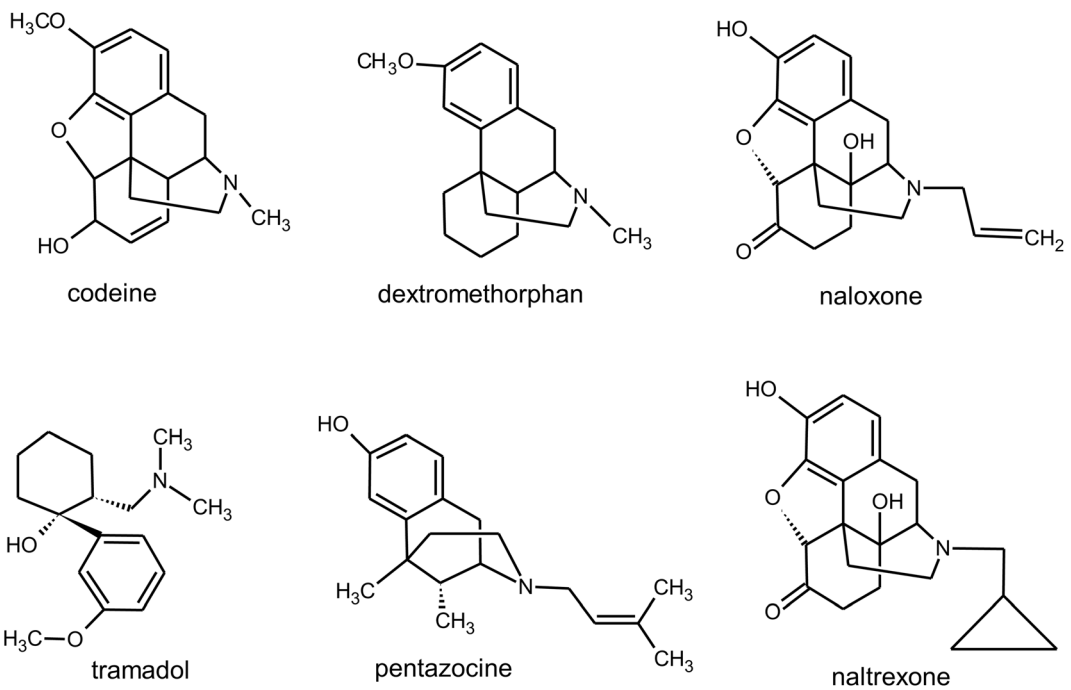

Fig. 1 Chemical structures of model opioid compounds.

while R_{mw} is the intercept and value of R_m extrapolated to pure water (0% methanol) as the mobile phase. The *x*-intercept of the linear plot (φ_0), also known as isocratic chromatographic hydrophobicity index (ICHI), gives the lipophilicity measure of the compound. The ICHI was used as a key parameter for subsequent validation analysis of the OL-C model.

2.3.2. Molecular descriptor computation. The molecular structures of the opioids were drawn with ACD/ChemSketch Software (version 8.0, Advanced Chemistry Development, Inc., Ontario, Canada), and molecular descriptors such as molecular weight, molar refractivity, molar volume, parachor, refractive index, surface tension, density and polarizability were calculated. *In vivo* biological parameters: blood–brain barrier (BBB) penetration, human intestinal absorption (HIA), and plasma protein binding (PPB), which are critical to the activity of these opioids, were computed with the preADMET software (http://preadmet.bmdrc.org).

2.3.3. Validity assessment of the OL-C performance as a biomimetic lipophilicity platform

2.3.3.1. Determination of limit of agreement of measurements. The degree of agreement in lipophilicity measurements by the two biomembrane models, *i.e.*, OL-C and ODS, was determined by the Bland–Altman plot.[10] A plot of the difference in ICHI values obtained on the OL-C and ODS surfaces was made on the *y*-axis against the mean of the measurements to generate a scatter plot that gives information on the average of all the measurement differences and the 95 percent limit of agreement of the measurements.[11]

2.3.3.2. Correlation analysis. The extent of correlation of lipophilicity index on OL-C with the pharmacokinetic parameters culled from existing literature, calculated molecular descriptors, and computed bioactivity-related parameters was determined using GraphPad Prism version 9 (GraphPad Software Inc., San Diego, CA, USA).

3. Results and discussion

The structures of the model opioids are shown in Fig. 1.

3.1. Chromatographic profiling

The retention attributes R_f and R_m of the compounds, determined at varied compositions of methanol/water mixture on two biomembrane models comprised of either OL-C or ODS, were determined. The linear regression plots of the chromatographic data of the six (6) model compounds on the two models are provided in Fig. 2. The OL-C model showed two clusters of characterization (codeine, naloxone, naltrexone in hydrophilic cluster and tramadol, pentazocine, dextromethorphan in lipophilic cluster), while the ODS did not show any evident cluster.

The regression parameters for the chromatographic evaluation are summarized in Table 1. All the compounds had greater lipophilicity indices on the ODS model compared to the OL-C model, such that some compounds characterized as lipophilic on ODS were classified as hydrophilic on OL-C, *e.g.*, codeine, naloxone and naltrexone. This reveals the difference in the complexities of the surface chemistry of these two biomembrane models and in the partition dynamics regulating retention behaviour. Also, difference in lipophilicity profiling on these two surfaces emphasizes the importance of the biomimetic feature of the lipid system as critical in impacting the retention behaviour, which is consistent with previous findings where ODS was shown to overestimate compound lipophilicity.[1]

3.2. Evaluation of the validity of the new OL-C film for accurate lipophilicity determination

3.2.1. Evaluation of limit of agreement. The Bland–Altman plot showing the paired difference between the two models against the mean of the paired models is shown in Fig. 3, in which the dashed horizontal lines represent the Limits of

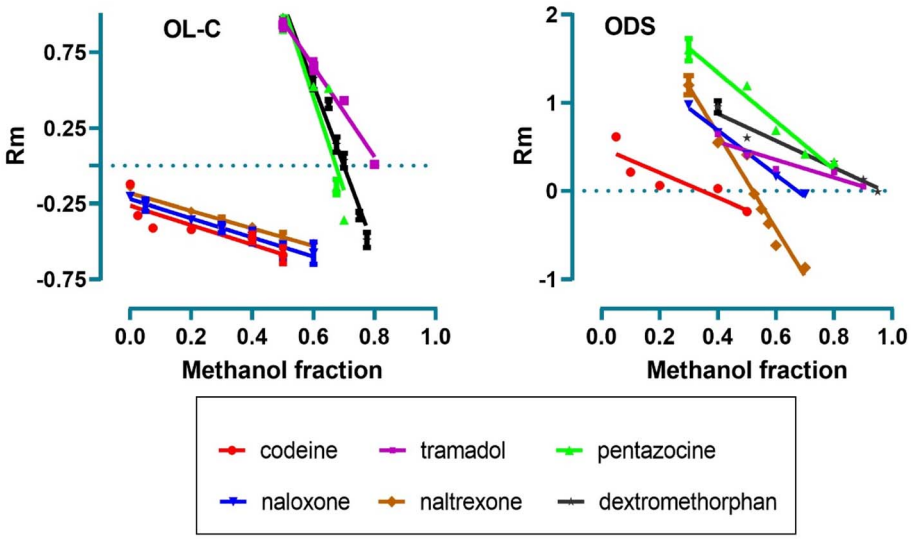

Fig. 2 Linear regression plots of R_m against methanol fraction for the six model compounds on the OL-C (left) and ODS (right) models.

Table 1 Regression parameters for the chromatographic evaluation on the two models (ODS and OL-C)[a]

	ODS				OL-C			
Compound	S	R_{mw}	φ_0	r^2	S	R_{mw}	φ_0	r^2
Codeine	−1.41 (±0.38)	0.49 (±0.11)	0.35	0.77	−0.64 (±0.18)	−0.26 (±0.06)	−0.41	0.71
Tramadol	−1.01 (±0.20)	0.96 (±0.13)	0.95	0.87	−2.99 (±0.25)	2.45 (±0.17)	0.82	0.98
Pentazocine	−2.75 (±0.30)	2.44 (±0.18)	0.89	0.95	−6.15 (±1.46)	4.14 (±0.92)	0.67	0.82
Naloxone	−2.52 (±0.13)	1.70 (±0.07)	0.67	0.99	−0.63 (±0.07)	−0.22 (±0.03)	−0.35	0.93
Naltrexone	−5.38 (±0.47)	2.80 (±0.25)	0.52	0.95	−0.58 (±0.06)	−0.18 (±0.03)	−0.31	0.94
Dextromethorphan	−1.53 (±0.18)	1.49 (±0.13)	0.97	0.95	−5.42 (±0.40)	3.79 (±0.27)	0.70	0.97

[a] S = slope; R_{mw} = basic lipophilicity parameter; φ_0 = isocratic chromatographic hydrophobicity index (ICHI); r^2 = coefficient of determination. S, R_{mw} and φ_0 are expressed as mean (± standard deviation) for two independent experiments.

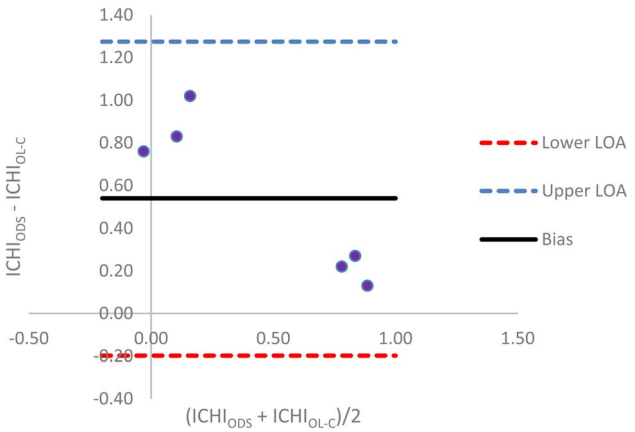

Fig. 3 Bland–Altman's plot of measurement difference against measurement average. Lower LOA = Lower Limit of Agreement, Upper LOA = Upper Limit of Agreement, bias = mean difference.

Agreement (LOA) (blue for upper LOA and red for lower LOA), while the black, solid, horizontal line represents the bias. The LOA quantifies the extent of variability within the paired differences of a measurement;[12] and the wider it is, the greater the dissimilarity of the measurements. The plot reveals a wide scatter without clustering of the data around the mean difference (bias) and has a wide LOA, indicating that the measurements on the two platforms are not comparable and thus the methods do not agree. This lack of agreement is expected, as OL-C and ODS are designed to represent different (biomimetic *vs.* hydrocarbon) partitioning environments and are therefore not intended to be interchangeable. Furthermore, the bias obtained for these two methods, which was 0.54, reveals an overestimation of compound lipophilicity by ODS and a poor limit of agreement between the methods.

3.2.2. Validation of model and quantitative structure–property relationship (QSPR). Quantitative structure–property relationship (QSPR) for the model compounds was assessed by correlational analysis of the experimental data with calculated molecular descriptors obtained from ACD/ChemSketch and with *in vivo* biological parameters. The pattern for the goodness of fit of correlation (r^2) for the molecular descriptors given in Table 2 shows that data from the OL-C model had better correlation with all the descriptors compared to the ODS model, which reached significance for molar volume as the molecular descriptor ($P < 0.01$).

3.2.3. Correlation with *in vivo* parameters. Fig. 4 shows the strength of correlation between the retention behaviours on OL-C and ODS and pharmacokinetic parameters: area under the plasma concentration–time curve (AUC) and maximum plasma concentration (C_{max}). OL-C correlated better than ODS for the two parameters.

Similarly, the pattern of correlation with *in vivo*-related plasma protein binding, intestinal absorption, and brain uptake is shown in Fig. 5. The OL-C model had higher correlation values for the retention parameters *versus* the *in vivo* parameters compared to the ODS model, indicating for the OL-C model an interaction of the drugs with the complex dissolved compounds in the oil, comparable to what obtains in the biological membrane. This reflects OL-C's better biomimetic properties compared to ODS. Hence, OL-C relative to ODS demonstrates better predictability of *in vivo* response from *in vitro* parameters.[13]

A leave-one-out sensitivity analysis to assess robustness was carried out (see SI data). Values on OL-C were consistently higher than those on ODS, except when the second data point was omitted from the C_{max} for OL-C. The leave-one-out sensitivity analysis showed that exclusion of tramadol data profoundly modified the outcome of correlation analysis, especially on OL-C, more than it did on ODS. To account for this observation, we noted that tramadol has the highest C_{max} of all the model compounds, as well as the highest lipophilicity measure on OL-C, whereas dextromethorphan has the highest lipophilicity measure on ODS. Tramadol has a unique structural feature as the only compound devoid of any fused ring out of the six compounds (see Fig. 1). On OL-C, the lipophilicity values fit into two distinct clusters, with three compounds clearly classified as hydrophilic (negative values of ICHI) (Fig. 2). On ODS platform, however, all the six compounds have positive ICHI values without any distinct cluster of values. The greater sensitivity of lipophilicity correlation with C_{max} on OL-C could therefore arise from this main difference in the pattern of lipophilicity measure on the two platforms.

Olive oil has a rich composition of flavonoids, polyphenols, triacylglycerol, phytosterols, terpenes, *etc.*,[14] a variable fatty acid composition,[15] and large polar constituents of ricinoleic acid

Table 2 Values of calculated molecular descriptors and the goodness of fit for the correlation with lipophilicity indices from the two stationary models (ODS and OL-C)

S. no	Theoretical molecular descriptors	Model compounds						Coefficient of determination (r^2) for correlation of ICHI with molecular descriptors	
		Codeine	Tramadol	Pentazocine	Naloxone	Naltrexone	Dextromethorphan	ODS	OL-C
1	Molecular weight (g)	299.4	263.4	285.4	327.4	341.4	271.4	0.476	0.742
2	Molar refractivity (cm^3)	82.85	77.96	88.2	87.31	90.11	81.76	0.142	0.264
3	Molar volume (cm^3)	222.6	251.3	275.2	228.1	230.8	243.8	0.545	0.669
4	Parachor (cm^3)	620.8	630.9	681.3	664.7	684.1	631.2	0.0002	0.0284
5	Refractive index	1.666	1.532	1.553	1.691	1.709	1.585	0.666	0.916
6	Surface tension (dyne cm^{-1})	60.5	39.6	37.5	72.0	77.1	44.9	0.561	0.845
7	Density (g cm^{-3})	1.34	1.047	1.037	1.43	1.47	1.11	0.653	0.914
8	Polarizability $\times 10^{-24}$ cm^3	32.84	30.9	34.96	34.61	35.72	32.41	0.142	0.264

(90%), amidst other fatty acids.[16] This provides the basis for the presumed amphiphilic properties of OL-C surface relative to pure hydrocarbon represented by ODS. The amphiphilicity of the OL-C surface facilitates the wettability,[17] and influences the retention behaviour of the solute in a similar manner to physiological settings.

3.2.4. Limitation of the study. A large sample size is often essential in validating model performance, in order to ensure prediction accuracy and reliability,[18] as inadequate data size makes a model unstable, imprecise in predictive performance, and unfit for use.[19] Owing to the difficulty in obtaining "opioids", which are controlled drugs, only a limited number of them could be sourced for this study. We, therefore,

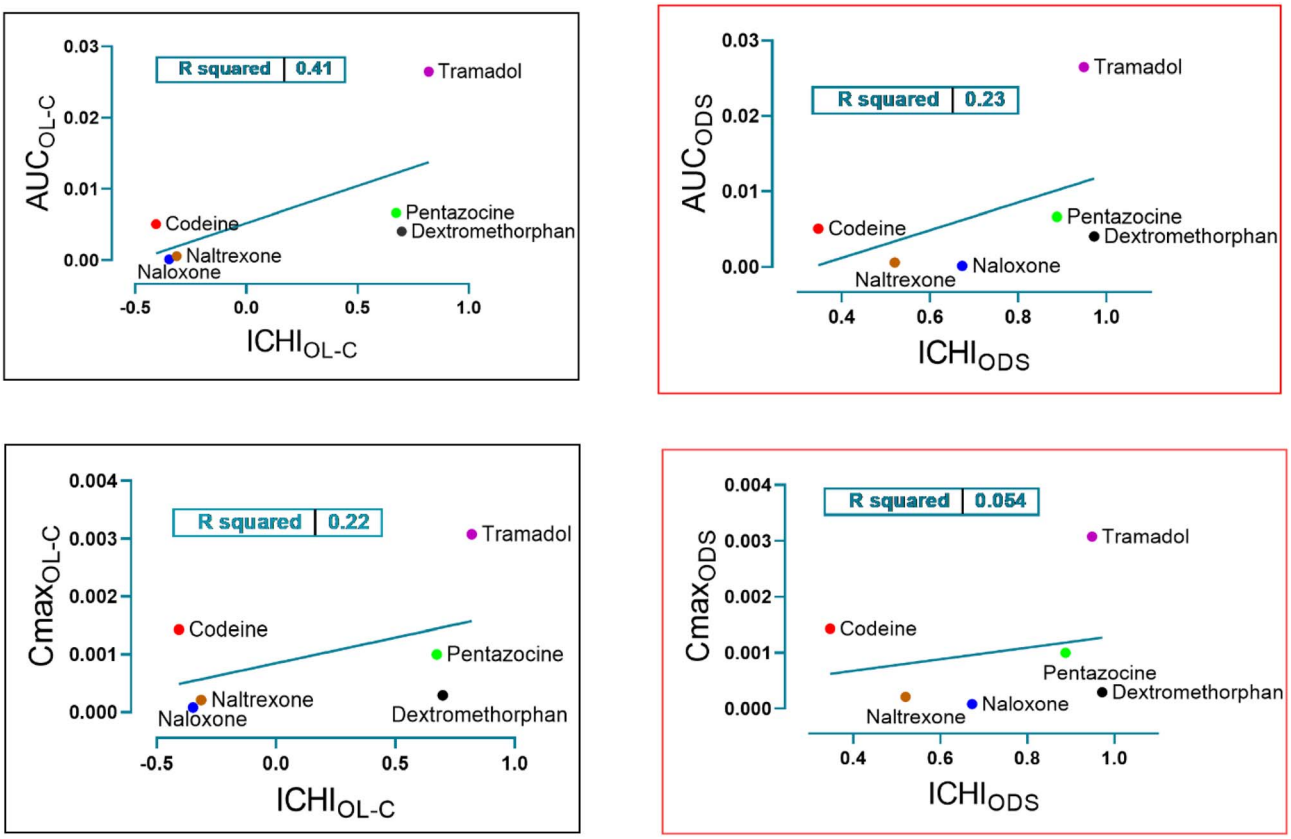

Fig. 4 Correlation plots of lipophilicity indices for the two models (ODS and OL-C) *versus* pharmacokinetic parameters (AUC and C_{max}), showing the goodness of correlation (R^2).

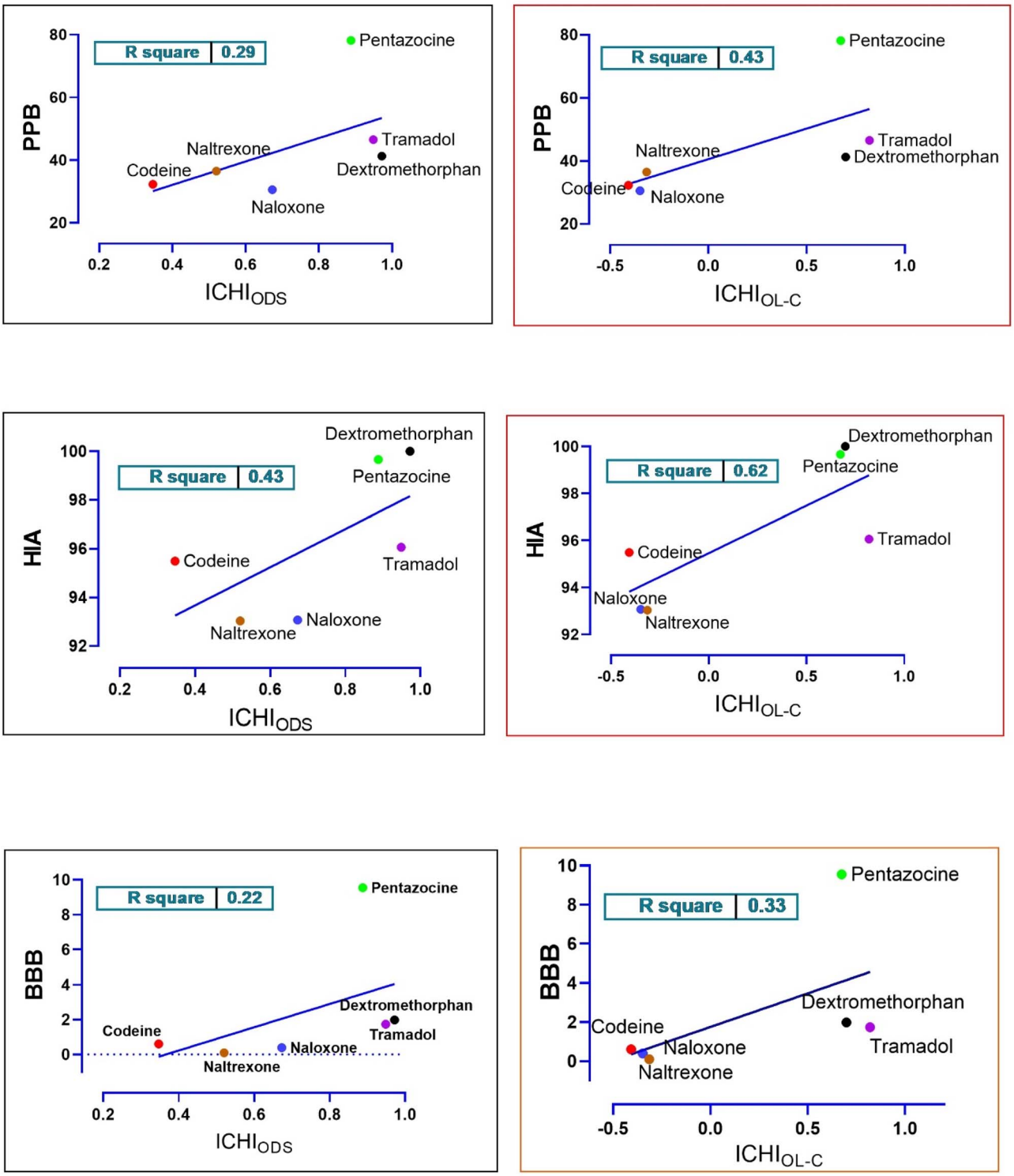

Fig. 5 Correlation plots of lipophilicity indices obtained from the two model platforms (ODS and OL-C) *versus* bioactivity-related parameters, showing goodness of correlation (R^2). PPB = Plasma Protein Binding, HIA = Human Intestinal Absorption, BBB = Blood–Brain Barrier.

acknowledge that the limited number of tested compounds (only six opioids) in this study was a limitation and plan to undertake in the future a broader validation across different chemical classes. Nevertheless, studies with small sample sizes are indeed useful in revealing useful patterns and providing proof of concept that could justify as well as guide the design of further studies with larger sample sizes.[20] Our future study will also examine variations in the power of the molecular descriptors to predict lipophilicity, as well as consider comparing OL-C with other biomimetic stationary phases reported in the

literature. The findings of this study have helped to demonstrate, at least, preliminarily, that the OL-C has significant potential as a more accurate platform than the ODS and can, therefore, be further extended to compounds of diverse pharmacophores.

4. Conclusion

The retention behaviour of the opioid drugs on OL-C platform compared to ODS shows better correlation with their pharmacokinetic properties, molecular descriptors, as well as their intestinal absorption, blood–brain barrier (BBB) uptake and plasma protein binding. This study thus validates the OL-C model as a better biomembrane model than the ODS. This platform will be extended in the future to evaluate a larger library of compounds with diverse pharmacophores to further establish its versatility, including its potential for high-throughput screening of compounds for lipophilicity.

Conflicts of interest

There are no conflicts to declare.

Data availability

Data for this paper, including raw datasets for calculating averages and standard deviations, are available at LJMU Data repository at https://doi.org/10.24377/LJMU.d.00000238.

Supplementary information (SI): data for leave-one-out sensitivity analysis. See DOI: https://doi.org/10.1039/d5ra05082k.

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