The Role of Molecular Descriptors as Screening Tools for the Pyridinealdoxime Methiodide Derivatives Biopharmaceutical Profile

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The paper presents an analysis of molecular descriptors with biological relevance for three series of pyridinealdoxime methiodide (2-PAM) analogues with potentially improved permeability characteristics. The biopharmaceutical profile is discussed by correlation with predicted solubility in various simulated biological fluids and permeability across several types of biological barrier. The data suggests that the increase in lipophilicity could be considered as a good approach in optimization of the low permeability profile determined by the highly polar structure, necessary for the reactivation of organophosphorous compounds inhibited cholinesterase.

Keywords: 2-PAM analogues, molecular descriptors, BCS

The use of oximes as reactivators for phosphorilated cholinesterase is the main antidotal approach in organophosphorous compounds (OPC) intoxication [1]. For their specific mechanism of action, the pronounced nucleophilicity and the high affinity for the enzyme are some of the most important characteristics [2]. From the biopharmaceutical point of view, the structural characteristics are transposed in a specific low permeability through the key biological barriers, such as gastro-intestinal membranes or blood-brain barrier (BBB) [3,4]. The biopharmaceutical classification system (BCS) was used since its introduction and especially the after regulatory official adoption as a bio-waiving tool, granting exception from the in-vivo evaluation based on solubility and permeability profiles of a drug substance included in a pharmaceutical dosage form [5,6]. The high-low definitions of the four BCS classes have become also an important screening tool for selection and development of new molecular entities with potential therapeutic use. The fact that a variable pharmacokinetic profile, driven by pH-dependent solubility and permeability limitations, will most probably lead to variability in drug exposure for the final therapeutic target has limited lately the development of drug candidates with low-solubility, low permeability characteristics (BCS class IV).

Starting from the BCS-based experimental data, describing both in-vitro and in-vivo solubility and permeability profile of a large number of pharmacologically-active entities, several rules of selection have been developed for the drug candidates. These rules include also a large and frequently, highly complex set of intervals for the values of several molecular descriptors, with clear correlation with specific absorption, distribution, metabolism and excretion (ADME) profiles [7-11].

The oximes reactivators of OPC-inhibited cholinesterase had a quaternary ammonium salt structure (mono or bis-pyridinium salts), the reason of a high solubility profile in all aqueous biological fluids and a limited permeability across phospholipidic membranes. Their appartenance to the class III of BCS (low permeability, high solubility) has limited their administration to the intravenous route [2]. Nevertheless, stability issues have been reported, completing furthermore the particular biopharmaceutical pattern [4].

Efforts have been made to limit the above mentioned disadvantage of low permeability. The most successful synthesis approaches have been oriented toward preservation of the pyridinium moiety, but substituted with long, saturated or aromatic chains [12].

The current paper presents synthetic analysis of molecular descriptors with biological relevance for three series of oximes designed for improved permeability characteristics.

Experimental part

Materials and methods

The oximes included in the current analysis were described in literature [12], as 2-pyridinealdoxime methiodide (2-PAM) analogues with potentially higher lipophilicity (see table 1). On the other hand, some of these 2, 3 or 4-PAM derivatives are among the few oximes reactivators of OPC inhibited cholinesterase for which data proving their BBB penetration are available [13].

The values of several molecular descriptors (topological polar surface area, T_PSA; number of H-bond donors, HBD; number of H-bond acceptors, HBA; number of freely rotatable bounds, FRB), and values of several BCS-correlated parameters (solubility in various simulated biological fluids, permeability across several types of biological membranes, octanol/water partition coefficient, LogP; Moriguchi model of octanol-water partition coefficient, MLogP; distribution coefficient at a specific *p*H, LogD; acid and base ionization constants, pKa or pKb) have been calculated using ADMET Predictor software, version 5.0.0012, Simulation Plus Inc.. For some of the most representative chemical structures of each series of oximes, the quantitative predictions of penetrations across BBB or Caco-II cells and partition coefficient across skin have been performed using the on-line version of PreADMET software [14,15]. Marvin was used for drawing, displaying and characterizing chemical structures, Marvin version number 5.3.8., 2010 (http://www.chemaxon.com).

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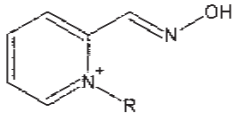
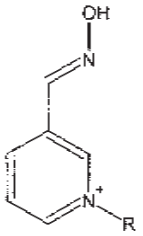
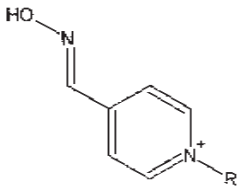
Table 1
DESCRIPTION OF THE THREE SERIES OF OXIMES INCLUDED IN THE ANALYSIS

2-PAM Series	3-PAM Series	4-PAM Series	Substituent (R)	Molecular weight	Molecular volume (Å^3)
			H	137.16	126
			2-ethyl-hexyl	235.35	273
			2-phenylethyl	227.29	210
			3-phenylpropyl	241.31	231
			4-phenylbutyl	255.34	252
			benzyl	213.26	189
			butyl	179.24	189
			decyl	263.41	315
			ethyl	151.19	147
			hexyl	207.30	231
			isoamyl	193.27	210
			octadecyl	375.62	483
			octyl	235.35	273
			p-methylbenzyl	227.29	210
			p-tertbutylbenzyl	269.37	273

Series	Substituent (R)	FRB	HBD	HBA	Acidic pKa	Basic pKa	MlogP	logP	logD pH=7.4
2-PAM	-H	0	1	2	7.87	-7.76	0.47	-	-3.01
	-2-ethyl-hexyl	6	1	2	8.37	-5.68	2.53	0.22	-0.66
	-2-phenylethyl	3	1	2	8.14	-6.3	2.29	-	-1.50
	-3-phenylpropyl	4	1	2	8.22	-6.05	2.54	-	-1.21
	-4-phenylbutyl	5	1	2	8.28	-5.85	2.79	-	-0.83
	-benzyl	2	1	2	8.03	-6.57	2.02	-	-1.66
	-butyl	3	1	2	8.14	-6.64	1.43	-	-2.15
	-decyl	9	1	2	8.44	-5.43	3.04	1.47	0.47
	-ethyl	1	1	2	7.93	-7.42	0.81	-	-2.86
	-hexyl	5	1	2	8.27	-6.11	2.00	-	-1.33
	-isoamyl	3	1	2	8.21	-6.36	1.72	-	-1.87
	-octadecyl	17	1	2	8.59	-4.72	**4.85**	**5.20**	3.99
	-octyl	7	1	2	8.37	-5.72	2.53	0.44	-0.44
	-p-	2	1	2	8.07	-6.43	2.29	-	-1.31
	-p-	3	1	2	8.21	-5.87	3.03	0.48	-0.28
3-PAM	-H	0	1	2	9.18	-6.38	0.47	-	-3.57
	-2-ethyl-hexyl	6	1	2	9.63	-4.32	2.53	0.14	-1.20
	-2-phenylethyl	3	1	2	9.38	-4.86	2.29	-	-1.98
	-3-phenylpropyl	4	1	2	9.45	-4.64	2.54	-	-1.69
	-4-phenylbutyl	5	1	2	9.51	-4.45	2.79	-	-1.32
	-benzyl	2	1	2	9.29	-5.11	2.02	-	-2.17
	-butyl	3	1	2	9.43	-5.26	1.43	-	-2.63
	-decyl	9	1	2	9.68	-4.07	3.04	1.37	-0.23
	-ethyl	1	1	2	9.26	-6.04	0.81	-	-3.38
	-hexyl	5	1	2	9.55	-4.74	2.00	-	-1.84
	-isoamyl	3	1	2	9.49	-4.97	1.72	-	-2.33
	-octadecyl	17	1	2	9.81	-3.39	**4.85**	**5.06**	2.81
	-octyl	7	1	2	9.63	-4.36	2.53	0.35	-1.04
	-p-	2	1	2	9.32	-4.98	2.29	-	-1.85
	-p-	3	1	2	9.44	-4.42	3.03	0.40	-0.87
4-PAM	-H	0	1	2	8.55	-6.94	0.47	-	-3.34
	-2-ethyl-hexyl	6	1	2	9.03	-5	2.53	0.27	-0.97
	-2-phenylethyl	3	1	2	8.83	-5.53	2.29	-	-1.76
	-3-phenylpropyl	4	1	2	8.89	-5.32	2.54	-	-1.47
	-4-phenylbutyl	5	1	2	8.94	-5.14	2.79	-	-1.12
	-benzyl	2	1	2	8.75	-5.76	2.02	-	-1.95
	-butyl	3	1	2	8.82	-5.89	1.43	-	-2.41
	-decyl	9	1	2	9.09	-4.75	3.04	1.45	0.03
	-ethyl	1	1	2	8.64	-6.62	0.81	-	-3.15
	-hexyl	5	1	2	8.94	-5.39	2.00	-	-1.63
	-isoamyl	3	1	2	8.88	-5.62	1.72	-	-2.10
	-octadecyl	17	1	2	9.23	-4.09	**4.85**	**5.14**	3.34
	-octyl	7	1	2	9.02	-5.02	2.53	0.45	-0.81
	-p-	2	1	2	8.79	-5.63	2.29	-	-1.62
	-p-	3	1	2	8.92	-5.09	3.03	0.55	-0.60

Table 2
THE CALCULATED VALUES OF MOLECULAR DESCRIPTORS FOR THE THREE SERIES OF OXIMES

Results and discussions

The analysis of several pharmacokinetic parameters and of molecular descriptors values for large series of compounds, mainly drugs and toxicants, have generated and validated several rules of correlations. These relationships are currently used mainly for discarding the chemical structures with potential limitation in solubility and permeability parameters, describing the concept of "developability".

It concerns the passive diffusion processes across the biological membranes, for oral absorption, the molecular weight is limited to a value 500, while the BBB penetrability

further limits this parameter to the interval 150 - 450 [7,8,10]. Nevertheless, LogP values are also considered, although this parameter seems to display a highly complex (frequently sigmoidal) relation with permeated quantities across lipophilic structure [16,17]. For intestinal absorption, LogP values lower than 5 are requested, and the limit drops to 2.5 for BBB permeability [7,9,11].

The results of prediction for several molecular descriptors relevant for both in-vivo absorption and distribution patterns are presented in table 2.

For the considered oxime structures, it seems that long side chains induce a considerable increase of LogP, this parameter overpassing even the intestinal absorption limit (5.20 for 2-PAM-octadecyl, 5.06 for 3-PAM-octadecyl and 5.14 for 4-PAM-octadecyl). The negative values of LogP are preserved, except for octyl, decyl, octadecyl, p-tertbutylbenzyl and 2-ethyl-hexyl derivatives. If one considers the partition at the physiological pH of 7.4 (LogD), a positive value is predicted only for the octadecyl derivative.

The roles of HBD and HBA have been postulated in [10], in the so called rule of five, correlating bioavailability problems with HBD values higher than 5 and HBA values higher than 10. This set of rules has been further adapted for highly specialized barriers, such as BBB, where intervals of 0-2 and 2-4, respectively, are considered as optimal for an acceptable permeation profile. This set of rules is fulfilled by all analyzed structures, except for the highly lipophilic octadecyl derivatives (due to their LogP values higher than 5, MLogP higher than 4.15, respectively).

The T_PSA is limited to values lower than 120Å for digestive absorption and lower than 60-70Å for BBB permeability. The predicted value for this molecular descriptor in case of all considered structures is 36.47Å, indicating favourable penetration parameters. A graphical representation of T_PSA, molecular weight and LogP values is presented in figure1, suggesting the existence of two extreme sub-groups: the phenyl-alkyl derivatives with polarity-limited permeability and octadecyl derivatives, with potentially, lypophilicity-limited solubility. In fact, the two subgroups are potentially belonging to different BCS classes (III and II, respectively).

The prediction of solubility and permeability further illustrates the impact of the nature of the side-chain for the oxime structures. The long saturated alkyl chains determined a net increase of predicted permeability across biological membranes, with a potential decrease of solubility. This fact is further supported by the solubility pattern in the physiologically relevant simulated fluids. Their compositions have been developed and optimized by the group of Dressman JE. [5,6], with the goal of simulating the properties of physiological gastric and intestinal secretions, on both fast and fed state (identified as FaSSGF, FeSSGF, FaSSIF and FeSSIF), in terms of pH, ionic strength, surface tension or lipid content. There is a strong correlation between the solubility parameters and partition / distribution coefficient, imposed by the fact that a higher lipophilicity will request a high quantity of tensioactive substance (in this case, of endogenous origin, e.g. sodium taurocholate) in order to dissolve and become available for the passive diffusion processes. This fact leaded to good linear correlations of predicted values of LogP and logarithm of solubility in FaSSGF (R2=0.994, RMSE=0.074), FaSSIF (R2=0.88, RMSE=0.326) and FeSSIF (R2=0.90, RMSE=0.284) (fig.2).

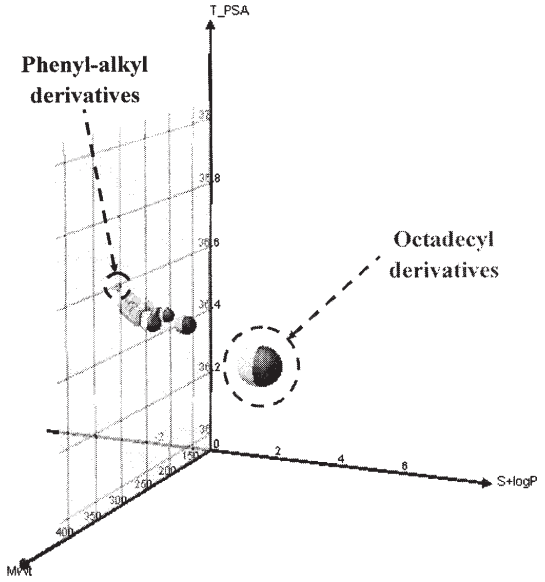


Fig.1 Three-dimensional representation of calculated molecular descriptors influencing the permeability across blood-brain barrier (T_PSA – topological polar surface area, Å^2; MWt – molecular weight; S+logP – predicted n-octanol/water partition coefficient)

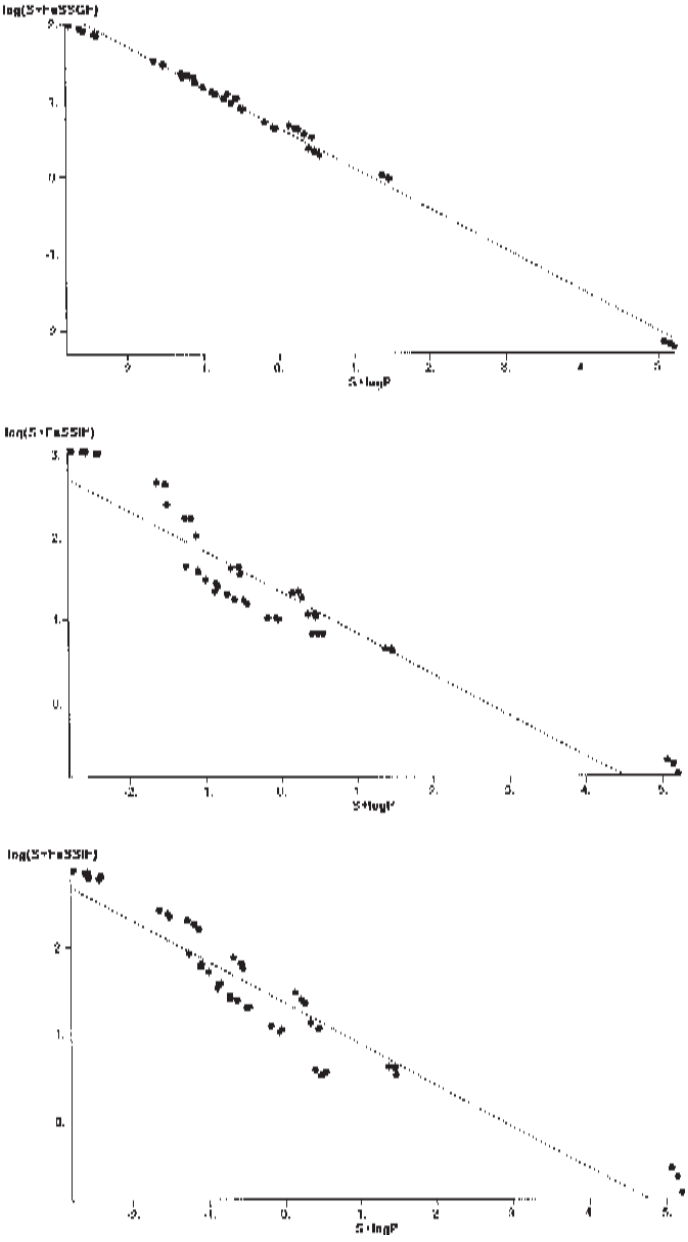


Fig.2. Correlation between predicted values of LogP and solubility in simulated gastro-intestinal fluids (mg/mL, logarithmic scale)

Table 3
THE PREDICTED SOLUBILITY AND PERMEABILITY CHARACTERISTICS FOR THE PAM DERIVATIVES

Series	Substituent (R)	FaSSGF (mg/ml)	FaSSIF (mg/ml)	FeSSIF (mg/ml)	Peff (cm/s x 10^{-4})	DiffCoef (cm^2/s x 10^{-5})	MDCK (cm/s x 10^{-7})	Pcornea (cm/s x 10^{-7})
2-PAM	-H	80.14	1084.50	613.59	0.29	1.29	94.23	240.84
	-2-ethyl-hexyl	4.31	21.40	24.67	0.97	0.82	80.45	387.82
	-2-phenylethyl	12.56	26.76	36.88	0.82	0.95	97.51	446.10
	-3-phenylpropyl	7.84	16.60	19.58	0.96	0.90	93.46	454.84
	-4-phenylbutyl	4.43	10.20	10.39	1.09	0.86	81.84	462.85
	-benzyl	16.88	38.92	59.02	0.71	1.01	93.43	436.65
	-butyl	29.37	433.47	236.78	0.50	1.01	84.00	313.57
	-decyl	0.92	4.14	3.31	1.24	0.75	64.13	411.39
	-ethyl	68.51	1052.76	639.53	0.34	1.18	91.96	270.68
	-hexyl	10.64	43.37	63.96	0.71	0.90	76.63	350.72
	-isoamyl	20.76	166.93	181.68	0.55	0.95	79.22	334.55
	-octadecyl	**0.01**	**0.13**	**0.15**	**2.54**	**0.58**	**46.34**	**498.33**
	-octyl	3.29	11.54	11.23	0.95	0.82	70.01	383.00
	-p-methylbenzyl	10.47	19.94	24.75	0.82	0.95	79.92	447.10
	-p-tertbutylbenzyl	2.07	6.73	3.29	1.13	0.82	73.76	487.85
3-PAM	-H	93.63	1086.99	749.62	0.28	1.29	80.27	212.01
	-2-ethyl-hexyl	4.67	20.70	28.91	0.96	0.82	72.97	352.58
	-2-phenylethyl	14.71	29.59	50.85	0.83	0.95	84.94	429.32
	-3-phenylpropyl	9.10	17.13	24.17	0.96	0.90	86.73	439.27
	-4-phenylbutyl	5.11	10.31	12.16	1.09	0.86	71.90	448.42
	-benzyl	20.11	43.67	82.76	0.71	1.01	87.26	417.90
	-butyl	32.77	463.84	262.95	0.51	1.01	74.21	280.26
	-decyl	1.02	4.36	4.21	1.24	0.75	59.47	376.10
	-ethyl	79.67	1067.91	709.26	0.34	1.18	78.83	242.30
	-hexyl	11.77	41.60	74.63	0.71	0.90	69.11	315.40
	-isoamyl	22.45	168.41	199.68	0.55	0.95	70.42	300.61
	-octadecyl	**0.01**	**0.19**	**0.28**	**2.55**	**0.58**	**44.40**	**468.87**
	-octyl	3.64	11.34	13.21	0.95	0.82	64.12	347.22
	-p-methylbenzyl	12.68	21.56	33.37	0.81	0.95	78.55	430.85
	-p-tertbutylbenzyl	2.31	6.74	3.88	1.12	0.82	73.54	463.24
4-PAM	-H	85.33	1091.57	708.90	0.26	1.29	77.98	263.30
	-2-ethyl-hexyl	4.15	17.89	22.18	0.91	0.82	67.38	411.70
	-2-phenylethyl	12.22	24.87	37.47	0.78	0.95	82.47	521.13
	-3-phenylpropyl	7.57	15.52	19.95	0.91	0.90	80.81	530.73
	-4-phenylbutyl	4.29	9.90	11.06	1.04	0.86	70.65	538.29
	-benzyl	16.86	37.09	62.74	0.68	1.01	82.25	509.14
	-butyl	29.43	250.58	218.18	0.47	1.01	68.68	334.57
	-decyl	0.95	4.37	4.00	1.18	0.75	55.40	432.29
	-ethyl	72.03	1035.65	595.35	0.32	1.18	73.57	299.66
	-hexyl	10.64	35.28	56.09	0.67	0.90	63.94	369.15
	-isoamyl	19.86	103.90	159.18	0.51	0.95	65.07	356.36
	-octadecyl	**0.01**	**0.17**	**0.22**	**2.49**	**0.58**	**42.27**	**537.08**
	-octyl	3.33	10.66	11.50	0.90	0.82	59.49	401.75
	-p-methylbenzyl	10.57	19.63	27.38	0.77	0.95	71.22	521.69
	-p-tertbutylbenzyl	1.91	6.61	3.65	1.07	0.82	66.97	559.76

Note: FaSSGF - solubility in fasted state simulated gastric fluid; FaSSIF - solubility in fasted state simulated intestinal fluid; FeSSIF - solubility in fed state simulated intestinal fluid; Peff - human jejunal effective permeability; DiffCoeff - molecular diffusion coefficient in water; MDCK - apparent MDCK COS permeability; Pcornea - permeability through rabbit cornea.

In terms of predicted BBB permeability, the ADMET Predictor software provides only qualitative predictions (high – low). All the considered structures were evaluated as high BBB-permeable, although the above analysis suggested large differences within each subgroup. The on-line version of PreADMET software provides quantitative evaluations of LogBB, defined as the logarithm of blood to plasma concentration ratio. For example, in the case of the octadecyl derivatives of 2-PAM, the value of LogBB is 1.18, compared to -0.39 of 2-PAM. It seems plausible that for all the considered compounds, the criterion of LogBB higher than -1 for significant central nervous system penetration [20, 34] is fulfilled. But limited in-vivo data is available, supporting this speculation. In [13] it was reported a brain concentration of 30% from the blood level for the n-octyl derivative of 4-PAM (known also as 4-PAO, SPK-3 or OPAB), corresponding to a LogBB value of-0.52, compared to the predicted -1.60. Frequently, the main reason of lack of correlation between the predicted and experimental values of permeated fractions is the interference of several transporters. Especially for highly specialized biological barriers such as BBB, the role of the processes of passive diffusion, driven by both lipophilicity and concentration gradients, is overwhelmed by multitude of active or passive transport systems available at the site of penetration. It is the case of the above mentioned 4-PAO compound, a possible substrate for the carnitine/organic cation transporter 2 (OCTN2) [13]. Also, the involvement of specific transporters must be considered as explanations for the permeability of other highly polar oximes, although currently only the role of stress in altering the permeability through BBB is clearly documented. But, a highly relevant fact to be considered in the design of potentially new reactivators of OPC inhibited cholinesterase is the changes induced in the distribution profile, with possible safety concerns [4].

Conclusions

The calculated molecular descriptors for three series of hydrophobic PAM-derivatives illustrate the influence of

chemical structure on their biopharmaceutical profile. The increase in lipophilicity could be considered as a good approach in optimization of the low permeability profile determined by their highly polar structure, necessary for the specific mechanism of action. Nevertheless, it seems plausible that the high values of n-octanol – water partition coefficient will also trigger a bioavailability drawback, by the dependency on *p*H and tensioactive substance of absorption process at the intestinal level. This idea is supported by the good correlation of the predicted values of solubility in the presence of endogenous tensioactive (simulated fluids) and LogP. The predicted high permeability through BBB further raises the question of whether or not this will lead to more pronounces expression of the oximes pharmacotoxiological profile.

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